Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26 (6) 1115 – 1131 (2022)
www.ejabf.journals.ekb.eg

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

Rania O. Barakat, Mahmoud M. El-Mezayen*, Abd-Elhakim, E. El-Gamal, Abdel-Ghany, A. Soliman
National Institute of Oceanography and Fisheries, Aquaculture Division, NIOF, Egypt
***Corresponding Author:** m.elmezayen@gmail.com

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

Keywords:
Oreochromis niloticu,
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-serw 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 $\pm$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 mimcs 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 (600ml of 95% alcohol +300ml formaldehyde + 100ml 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 I mol /L of HCL and 1 mol/l of NaOH. The variation of pH in each measuring rarely exceeded $\pm$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 5.5mg/ 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)= $W_2 - W_1$

Weight gain rate (WGR) = $W_2 - W_1 / W_1$

where W_1,W_2 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:

condition factors= (W/L^3) x100

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 freezed 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 decribed 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 $\pm$ 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). This

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 phosphates (Alk phosphates)

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})

Parameter	**pH**							
Time (hours)	**Control**	**4**	**5.5**	**6.5**	**7.5**	**8.5**	**9.5**	**10.5**
25	10	100	15	10	8	11	15	100
50	13	-	20	20	12	13	30	-
75	10	-	10	15	10	10	25	-
96	4	-	10	5	5	9	20	-
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

Parameter	**pH (Avg±SD)**																	
Time (hours)	**Control**			**5.5**			**6.5**			**7.5**			**8.5**			**9.5**		
Hatching rate %	71.66	±	0.12	35.00	±	1.63	41.33	±	1.24	71.00	±	0.81	67.66	±	1.24	34.00	±	2.34
Abnormalities rate %	24.66	±	2.05	61.33	±	1.24	54.66	±	1.69	27.00	±	0.82	31.00	±	0.81	61.66	±	2.44
Incubation time (hrs)	22.33	±	2.05	32.33	±	2.05	36.00	±	0.18	32.00	±	2.16	33.00	±	1.63	46.00	±	2.16

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

Variable	**pH**					
	Control	**5.5**	**6.5**	**7.5**	**8.5**	**9.5**
Mean of length (mm)	1.59 (+ 0.25)	-	1.41 (+ 0.13)	1.64 (+0.21)	1.65 (+0.21)	1.55 (+0.17)
Mean of weight (mg)	0.051 (+0.021)	-	0.036 (+0.009)	0.053 (+0.01)	0.066 (+0.01)	0.055 (+0.01)
Survival rate %	77.33 (+2.52)	7.00 (+1.73)	50.67 (+ 4.04)	73.33 (+ 4.73)	68.67 (+3.51)	63.67 (+3.05)

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

Variable	pH					
	Control	**5.5**	**6.5**	**7.5**	**8.5**	**9.5**
Length (mm)	1.87 (±0.41)	-	1.65 (± 0.37)	2.16 (±0.79)	2.1 (±0.14)	1.78 (±0.21)
Weight (mg)	0.12 (±0.035)	-	0.083 (±0.017)	0.20 (±0.077)	0.13 (±0.045)	0.07 (±0.014)
Survival rate %	75 (±3.00)	-	65.33 (± 2.52)	72.00 (±1.00)	69.33 (±2.52)	64.33 (±3.06)
Condition factor	2.040 (±0.87)	-	1.602 (±1.39)	2.099 (±0.84)	1.635 (±0.87)	1.334 (±0.24)

Number of measured larvae in each treatment was 30; standard deviation in each treatment is put between brackets.

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

pH	**20 DPH**			**35 DPH**		
	Avg	**±**	**SD**	**Avg**	**±**	**SD**
Control	77.33	±	2.52^{a}	75.00	±	3.00^{a}
5.5	7.00	±	1.73^{e}	0.00	±	0.00^{e}
6.5	50.67	±	4.04^{d}	65.33	±	2.52^{cd}
7.5	73.33	±	4.73^{ab}	72.00	±	1.00^{ab}
8.5	68.67	±	3.51^{bc}	69.33	±	2.52^{bc}
9.5	63.67	±	3.05^{c}	64.33	±	3.06^{d}

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

Table 6. Influence of pH on the mean of secreted enzyme (µg/l) for larvae after 40 days of post- hatching

pH	Amylase			Alk phosphatase			Lipase		
	Avg	**±**	**SD**	**Avg**	**±**	**SD**	**Avg**	**±**	**SD**
control	10.20	±	0.36^{a}	1.82	±	0.02^{a}	7.67	±	0.61^{a}
5.5	1.50	±	0.10^{e}	0.14	±	0.02^{e}	1.67	±	0.32^{e}
6.5	5.50	±	0.10^{c}	0.81	±	0.01^{c}	6.27	±	0.06^{b}
7.5	8.27	±	0.21^{b}	0.90	±	0.02^{b}	7.17	±	0.15^{a}
8.5	4.27	±	0.25^{d}	0.22	±	0.03^{d}	4.53	±	0.25^{c}
9.5	1.70	±	0.10^{e}	0.04	±	0.02^{f}	2.47	±	0.15^{d}

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.

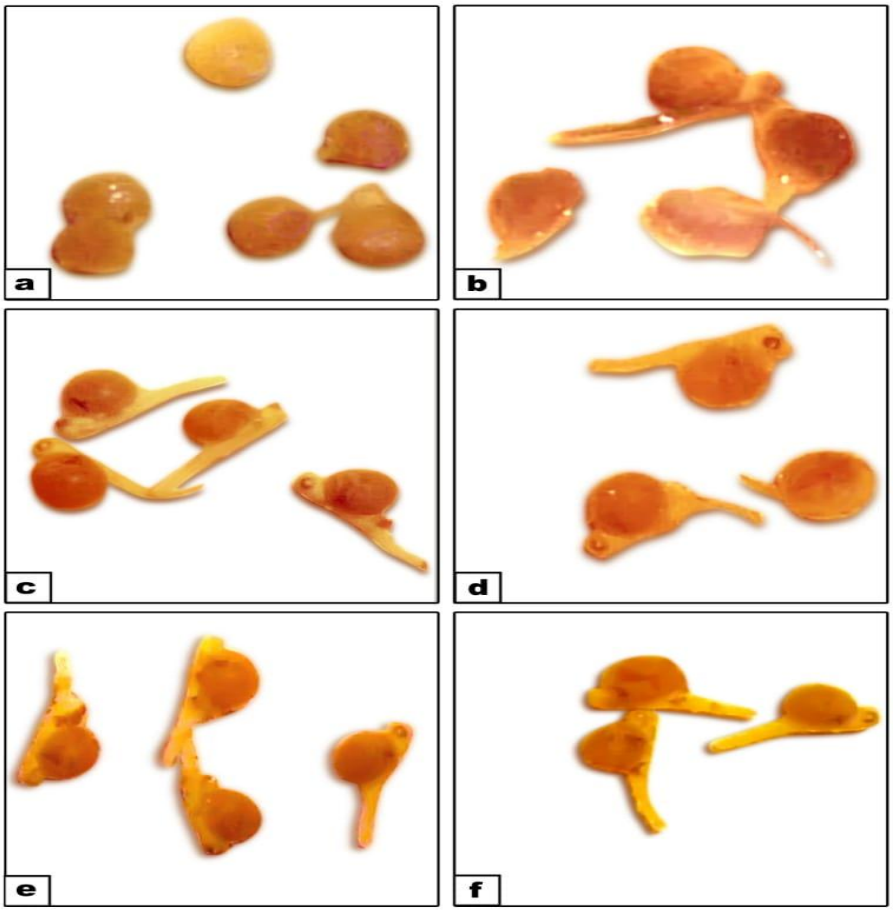

Fig. 1. Influence of different degrees of pH on fertilized eggs of early life stage larvae; the pictures were picked by a digital camera.

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

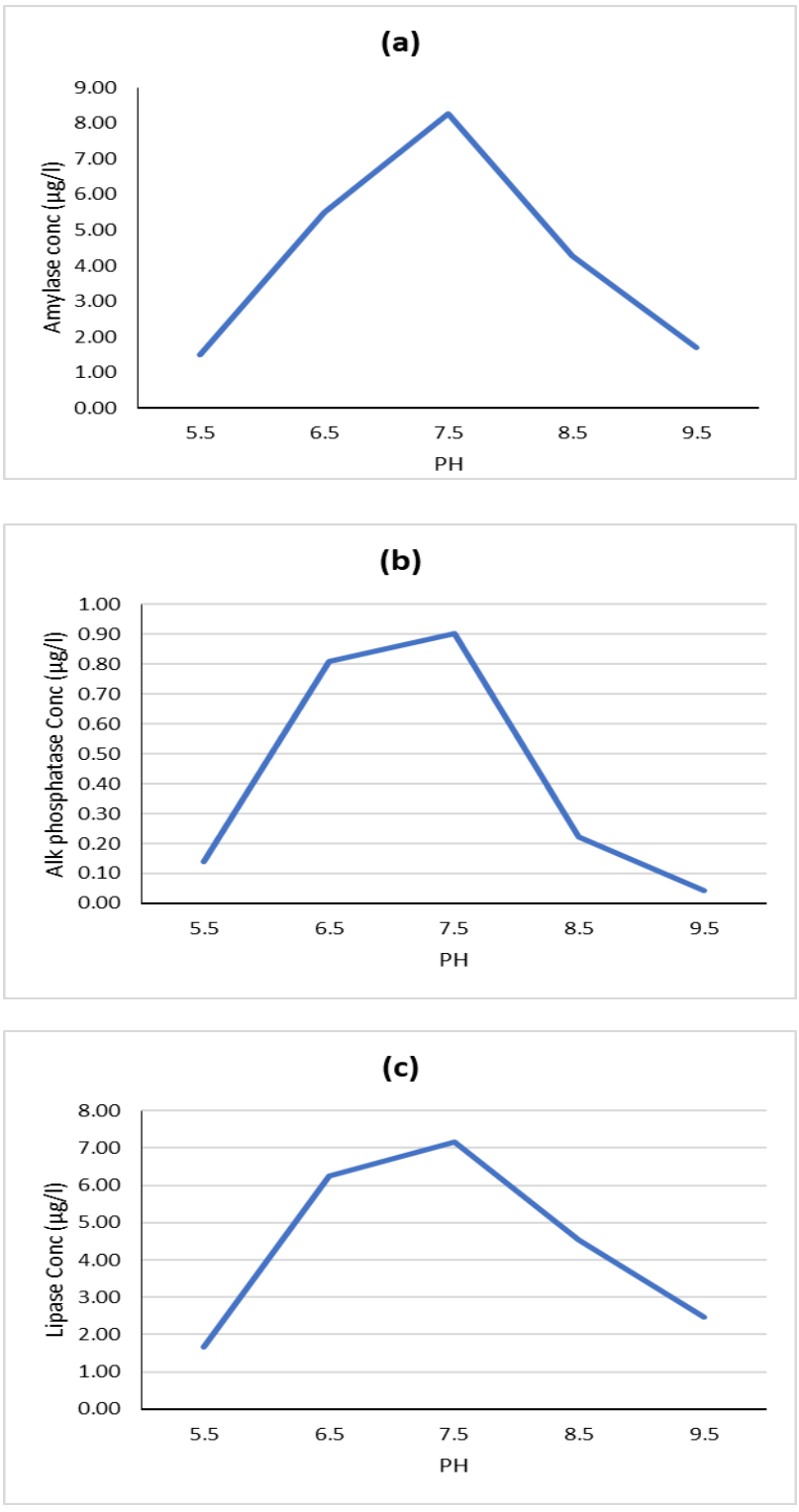

Fig. 2. The relation between the concentrations in (µg/l) of (***a***) Amylase (***b***) Alkaline phosphatase, and (***c***) Lipase enzymes and different degrees of pH values.

DISCUSSION

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 Chijioke (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 & Chijioke, 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 resechers 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 garriepinus*, 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 **Satyyaneson (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 woulld 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 $\pm$ 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 l0 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

Barakat, R.O.; El-Gamal, A. E; El-Mezayen, M. M.; El-Greasy, Z. A. and Sheha, M. A. (**2021**). Role of thyroxine hormone on eggs, thyroid gland development and growth performance of the monosex Nile tilapia, *Oreochromis niloticus* larvae. Egy. J. Aqua. Biol. Fish., 45: 253-269.

Beamish, R.J. (1976). Acidification of lakes in Canada by acid precipitation and resulting effects on fishes. Water, Air, Soil Poll., 6: 501-514.

Buritis, C.A. (1999). Tietz textbook of clinical chemistry, third ed. Saunders.

Buchet,V. ; Infantle, J. Z. and Cahu, G.l. (2000). Effect of lipid level in the compound diet on the development of the red drum *Sciaenops Ocellatus* larvae. Aquacul., 184: 339-347.

Cahu, C. and Infantle, J.L. (2001). Substitution of live food by formulated diets in marine fish larvae .Aquacul., 200:161-180.

Carrick, T. R. (1979). The effect of acid water on the hatching of Salmonid eggs. J. Fish. Bio., 14 :165-172.

De Silva, S.S. and Anderson, A.T. (1995). Fish nutrition in aquaculture. Chapman and Hall. London, UK.

Dillon, J.P.J; Yan, N.D. and Harvey, H. H. (1984). Acid deposition: Effects in aquatic ecosystems. Crit. Rev. env. Cont., 13: 167-194.

Béraud-Dufour, S.; Abderrahmani, A.; Noel, J.; Brau, F.; Waeber, G.; Mazella, J. and Coppola, T. (2010). Neurotensin is a regulator of insulin secretion in pancreatic beta-cells. Int. J. Bio & cell biol., 42: 1681-1688.

El-Greisy, Z.A. and El-Gamal, A. E. (2012). Monosex production of tilapia, *oreachromis niloticus* using different doses of 17 methyltestesterone with respect to the degree of sex stability after one year of treatment. Egy. J. Aquat. Res., 38: 59-66.

EL-Greisy, Z.A.; El-Gamal, A.E and Ahmed, N.A. (2016). Effect of prolonged ammonia toxicity on fertilized eggs, hatchability and size of newly hatched Larvae of Nite tilapia *Oreochromis niloticus.* Egy. J. Aquat. Res., 42: 215-222.

Feng, X.Y.; Zheng, J.S. and Wang, M. I. (2003). A study of the histochemistry on the digestive tract of the *Sebostes schlegeli* (J). J. Oceano. Univ. Qingdao., 33: 399- 404.

Golchinfar, N. F.; Zamanin, A.; Hajimora, d. A. and Madani, R. (2011). Assessment of digestive enzymes activity during the fry, development of Rainbow Trout, *Oncorhynchus miykiss*: from hatching to primary stages after yolk sac absorption. Iran. J. Fish. Sci., 10:403-414 .

Guan, H. H.; Pan, W. Z.; Chen, J.; Zhao, C.G. and Liu, W. (2006). Post- embryonic development of liver and pancreas and absorption of yolk in *Silurus Soldatovi* and *Silurus a sotus* and ft (J). J. Fish. Sci., 13: 460-464.

Hagenmaier, H. E. (1974) The hatching process of fish embryos IV. The enzymological properties of highly profied enzyme chorinase from hatching fluid of the rainbow Trout, *Salmo gairdneri* Rich Comp –Biochem. Physiol., 49:313-324.

Haines, T.A. (1981). Acid precipitation and its consequences for aquatic Ecosystems Review. Trans. Amer. Fish. Soc., 110: 669-707.

Haya, k. and Wainwood, B. A. (1981). Acid pH and chorinase activity of Atlantic Salmon eggs. Bull. Env. Cont. Tox., 27: 7-12.

Leurent, P.; Wkie, M.P.; Chevaliere, C. and Wood, C.M. (2000). The effect of highly alkalin water (9.5) on the the morphology and morphometry of chloride cells and pavement cell in gills of fresh water rainbow trout relationship to ionic transport and ammonie excretion. Con. J. Zool., 78: 307-319.

Menendez, R. (1976). Chronic effects of reduced PH brook trout (*Salvelinus Fontinalis*). J. Fish. Res. Board Cand., 33: 118-123.

Ndubuisi, U.C.; Chimezie, A.J.; Chinedu, U.C.; Chikwen, J.C. and Alexander, U. (2015). Effect of PH on the growth performance and survival rate of *Clarias gariepinus* fry. Int. J. Res. Biosci., 4: 14-20.

Nchedo, A.C. and chiJioke, O. G. (2012). Effect of PH on hatching success and Larval survival of African catfish (*Clarias gariepinus*). Nat. Sci., 10: 47-52.

Onucha, G.C and Nwadukwe, F.O. (1990). Influence of liquid petroleum refinery effluent on the hatching success of *Clarias gariepinus* (African Mid fish) eggs. Env. Eco., 8: 1201-1206.

Peterson, R.H.; Daye, P. G. and Metcalfe, V. (1980). Inhibition of Atlantic Salmon (*Salmo sular*) hatching at Low PH. Can. J. Fish. Aqua. Sci., 37: 770-774.

Ram, R.N. and Satyyanesan, A.G. (1985). Mercunic chloride Cythion and Ammonium sulfate induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish (J) *chamo puntactus* . Emir. Ecol., 3: 263-268.

Rask, M. (1983). The effect of low PH on perch, *perca fluviatilis*, L,I. Effect of Low PH on the development of eggs of perch. Ann. Zool. Fennici. 20: 73-76.

Reitan, H. k.; Kiorsvik, E. and Gjelle svik, D.R. (2001). Development of bile salt dependent Lipase in Larval turbot. J. Fish. Biol., 58: 737–745.

Ribeiro, L.; Zambonino-Infante, J. L.; Cahu, C. and Dinis, M. T. (1999). Development of digestive enzymes in Larvae of *Solea senegalensis* Kaup 1858.. Aquacult., 179: 465- 473.

Swarup, G. (1981). Selective dephosphorylation of protein containing phosphotyrosine by, alkaline-phosphatases. J. Biol. Chem., 256: 8197-8201.

Szezerbike, P; Mikoajezyk, T.; Sokoo Mikoajezyk , M. ; Socha , M.; Chyb, J. and Epler, P. (2008). The influence of Cadmium in Prussian Carp oocyte maturation development of eggs and hatching. Czec. J. Anim. Sci., 53: 36-44.

Trojnar, J. R. (1977). Egg hatchability and tolerance of brook trout. (*Salvelinus Fontinalis*) fry at low PH. J. Fish. Res. Board Can., 34: 575-579.

Watinab ,T. and Kiron,V. (1994).Prospects in larval fish diets . Aquacul., 124: 223–251.

Win Deen, E.S.; David, H.; Siglers, E. G. and Chavez, R. (1988). Development of a direct assay for alpha-amylase. Clin. Chem., 34: 2005-2008.

Wu, X. F.; Zhaon, J. B.; Qing, y.z. and Wu, C. (2007). Histological study the digestive system organogenesis of mandarin fish, *Siniperca chuatsi*. J. Zool. Res., 28:511-518.

Young, D. S. (1997). Effect of drugs on a clinical laboratory tests, fourth ed., Ann. Clin. Biochem., 34: 579-581.

Ye, J.S.; Chen.; Xiao, J. and Zhu, Y. Y. (2013). Influence of pH on survival, growth and activities of digestive enzyme of *Odontobuties obscures*. Adv. J. food sci. Tech., 5: 1234-1237.