

## Comparison Between Recirculating Aquaculture System (RAS) Ponds and Earthen Aquaculture Ponds in Overwintering Juveniles of the Nile Tilapia, *Oreochromis niloticus*

Hany Maher<sup>1</sup>, Ahmad M. Azab<sup>1\*</sup>, Ismail A. Radwan<sup>2</sup>, Mohamed Sh. Abu Husein<sup>1</sup>

<sup>1</sup>Zoology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt

<sup>2</sup>Egyptian Aquaculture Centre for training and applied research, Kafr El-Sheikh, Egypt

\*Corresponding author: [amazab2000@yahoo.com](mailto:amazab2000@yahoo.com)

### ARTICLE INFO

#### Article History:

Received: Feb. 15, 2023

Accepted: Feb. 24, 2023

Online: Feb. 28, 2023

#### Keywords:

Overwintering,  
Fish aquaculture,  
*Oreochromis niloticus*,  
Recycle Aquaculture  
system (RAS),  
Growth performance,  
Survival rates,  
Economic value

### ABSTRACT

The present study was carried out to compare the normal aquaculture in earthen ponds and the recirculating aquaculture system (RAS) in the overwintering of juvenile Nile tilapia, *Oreochromis niloticus* during the winter season. The overwintering experiment was carried out in 3 earthen ponds, each of 1 feddan (as replicates of a control group), and 5 concrete tanks (3\*8\*1 m<sup>3</sup> concrete tanks, as replicates of recirculating aquaculture system, RAS treatment group) at an intensive fish farm in EL-Hammoul province, Kafr EL-Sheikh Governorate, Egypt. The experiment extended from December 2019 to April 2020. Results showed that the highest values of final length, length gain, average daily length gain, final weight, weight gain, average daily weight gain and specific growth rate were recorded for fish fingerlings reared in the control earthen ponds. These growth performance parameters were significantly varied between all concrete ponds of the recycle aquaculture system (RAS) treatment and that of the control ponds. On the other hand, the best results of food conversion ratio, feed efficiency ratio, and protein efficiency ratio were estimated to fish reared in RAS treatment ponds. The mortality rate was very high for the control fingerlings where it reached 80.33%, while it recorded only 9.1% for RAS treatment fishes. The economic analysis showed a positive net income value for RAS treatment ponds and a negative net income value for earthen ponds. The present study recommended overwintering the fingerlings of the Nile tilapia, *O. niloticus* in RAS ponds with controlled water temperature to enhance the fish culture in Egypt and prevent the terrible mortality of fish.

### INTRODUCTION

The importance of aquaculture in the production of animal protein is evident, given the increase in the population and stagnation in production by natural fishing. The 70% increase in the production of the Nile tilapia (*Oreochromis niloticus*) over the past eight years has positively affected global freshwater aquaculture. In 2018, the production of tilapia was the third most farmed fish species globally, surpassing the production of common carp, *Cyprinus carpio*, and this expansion of production has led to a steady increase in the per capita consumption of tilapia (FAO, 2020).

The ideal temperature for tilapia is in the range of 26- 30°C. There is a decrease in feed consumption, which leads to a significant decrease in growth, when tilapia is bred at a suboptimal cold temperature of 22°C instead of the optimal temperature of 28 degrees Celsius. However, the temperature range at which feeding and voluntary movement stop, as

well as the lethal temperature, are significantly influenced by heredity and nutrition (Azaza *et al.*, 2008; Ma *et al.*, 2015; Correa *et al.*, 2017, 2018; Abdel-Ghany *et al.*, 2019).

Suboptimal cold temperatures have been reported to negatively affect the tilapia production worldwide. Along with a decrease in metabolic rate and growth, cold temperatures hinder the immunity of fish, which can lead to increased mortality or a variety of almost fatal consequences due to limited energy resources for the proper functioning of organs (Sun *et al.*, 1992; Dan & Little, 2000; Chang *et al.*, 2006; Ma *et al.*, 2015; Shi *et al.*, 2015; Nobrega *et al.*, 2019).

Studies have shown that the degree of thermal tolerance of the Nile tilapia depends on environmental influences, geographical distribution, fish history and genetic influences, as well as fish health and nutritional status (Siva *et al.*, 2002; Charo-Carissa *et al.*, 2005; Azaza *et al.*, 2008; Ma *et al.*, 2015; Abdel-Ghany *et al.*, 2019).

Fish naturally choose the habitat that is most suitable for their physiological requirements; accordingly, they move to deeper waters when the temperature drops or exceeds its ideal range (Dan & Little, 2000). Given that the water has a high specific heat, which makes it resistant to temperature change (Egna & Boyd, 2017), another winter production strategy is to adjust the depths and/ or dimensions of the pond and reservoir in order to enable fish move to deeper water when necessary to meet their thermal requirements (Abdel-Aal, 2008; Dan & Little, 2000; El-Sayed *et al.*, 1996).

In China, the recommended aquarium depth for tilapia production during winter is up to 2- 2.5m (Lin, 1991). In a study conducted on the Damietta branch on the Nile River in Egypt, one- to 2-meter deep ponds facilitated successful tilapia production at 38 to 13°C, supporting high growth performance and reducing mortality by 27 and 21.5%, respectively. In ponds with a depth of 3m, when water temperatures dropped to degrees between 13 and 5, fish avoided low temperatures by moving to the bottom of ponds, which led to a 21% decrease in mortality (El-Sayed *et al.*, 1996). In Taiwan, the use of deep ponds with wind shelters during winter was a common practice to prevent the occurrence of cold winds (Jobling, 1994).

Thus, the present study was carried out to compare the normal aquaculture in earthen ponds with the recirculating aquaculture system (RAS) in their effect on overwintering to the juvenile Nile tilapia, *Oreochromis niloticus* during the winter season.

## MATERIALS AND METHODS

### 1. Overwintering experimental design

The experiment of overwintering was carried out in earthen ponds as a control group and in the underground water inside the recirculating aquaculture system (RAS) concrete ponds as a treatment group.

#### 1.1. Earthen ponds

The control experimental group was carried out (for the second winter season, 2019-2020) in 3 earthen ponds on a personal fish farm, at Radif, El-Reyad Kafr EL-Sheikh Governorate (31°24'17.8"N 30°57'22.6"E). The ponds have the same area of about 1 feddan for each, with an approximately average depth of 1.25m and filled in through drainage 7.

### 1.2. Recirculating aquaculture system (RAS) ponds

The underground water recirculating aquaculture system (RAS) treatment was carried out at an intensive fish farm depending on circulated aquaculture system, located in EL-Hammoul province, Kafr EL-Sheikh governorate, Egypt.

The RAS farm consists of 24 concrete tanks (3 long x 8 x 1 m<sup>3</sup>) which were modified to match the water recirculation system requirements. All fish tanks were set in a greenhouse with diameters of 3X8 m. The tanks were arranged in two rows, each row having an irrigation channel with a drained channel in the middle. The experimental farm was completely described in **Abdel Hakim et al. (2016)**.

### 1.3. Experimental fish grouping and stocking

The experimental fish was the monosex of the Nile tilapia, *Oreochromis niloticus* fingerlings. The experiment extended from December 2019 to April 2020 (112 days). The experimental fish diet (floating pellets in RAS tanks and sinking pellets in earthen ponds) was of the commercial type (30% protein), purchased from the Zoo Control Industrial Company in the 6<sup>th</sup> October City.

The control group was carried out in three replicate earthen ponds (EP). They were stocked by 350000, 300000 and 250000 monosex fries of the Nile tilapia in pond 1 (EP<sub>1</sub>), pond 2 (EP<sub>2</sub>) and pond 3 (EP<sub>3</sub>), respectively. These fries were reared in ponds from September 2019 till December 2020. At the beginning of the overwintering experiment, they reached to the stage of fingerlings, with an average length of 5.16± 0.589cm and average weight of 4.49± 1.413g.

The treatment experiment group was carried out in five concrete ponds (CP) as underground water RAS replicates (CP<sub>1</sub>, CP<sub>2</sub>, CP<sub>3</sub>, CP<sub>4</sub> and CP<sub>5</sub>). Each concrete pond was stocked by 48000, 42000, 36000, 30000 and 24000, respectively, fingerlings monosex of the Nile tilapia, with an average length of 3.26±0.228cm and an average weight of 4.16±0.377g.

## 2. Fish growth parameters

Growth in length (**L** & **GL**), length gain (**LG**), daily length gain (**DLG**), growth in weight **W** & **GW**, total weight gain (**WG**), daily weight gain (**DWG**), specific growth rate (**SGR**) in the juveniles of *Oreochromis niloticus* were determined according to **Recker, (1975)** and **Castell and Tiews, (1980)** as follows:

$$\text{Length gain (cm/fish)} = \text{Final length (cm)} - \text{Initial length (cm)}$$

$$\text{Daily length gain (}\mu\text{/fish/day)} = \text{Total length gain (}\mu\text{)} / \text{Duration period (days)}$$

$$\text{Growth in length (\%)} = \{ \text{Length gain (cm/fish)} / \text{Initial length (cm)} \} \times 100$$

$$\text{Weight Gain (g/fish)} = \text{Final means weight (g)} - \text{Initial means weight (g)}$$

$$\text{Daily weight gain (mg /fish/day)} = \text{Total weight gain (mg)} / \text{Duration period}$$

$$\text{Growth in weight (\%)} = \{ \text{Weight gain (g/fish)} / \text{Initial means weight (g)} \} \times 100$$

$$\text{Specific growth rate (\% / day)} = \{ \text{Ln } W_f - \text{Ln } W_i \} * 100 / \text{Duration period}$$

Where, **Ln**= Natural log; **W<sub>f</sub>** = Final means weight of fish (g), **W<sub>i</sub>** = Initial means weight (g)

## 3. Feed utilization parameters

The total feed intake (**FI**), food conversion ratio (**FCR**); feed efficiency (**FE**), protein intake (**PI**) and protein efficiency ratio (**PER**) were calculated from the following equations:

$$\text{Feed intake (g)} = \sum \{ \text{Monthly average fish weight} * (\text{daily feeding rate} * 25 \text{ days}) \}$$

**Food conversion ratio = Feed intake (g) / Total weight gain (g)**

**Feed efficiency = Weight gain (g) / Feed intake (g)**

**Protein intake = Feed intake (g) \* Protein% in the diet/100**

**Protein efficiency ratio = Total weight gain (g) / Protein intake (g)**

#### 4. Mortality rate (MR, %)

The survival rate (SR, %) was calculated by the following equation:

$$\text{Mortality rate (\%)} = (N_i - N_f) / N_i * 100$$

Where,  $N_f$  = Number of fish survived at the end of the experiment, and

$N_i$  = Number of fish stocked at the start of the experiment

#### 5. Economic evaluation

Total costs were calculated by the following equation:

$$\text{Total costs} = \text{Feed costs (LE)} + \text{Fish fry cost (LE)} + \text{Operation cost (LE)}$$

Operation costs include workers, electricity, service...etc. All experimental diet costs, fish fingerlings cost and operation cost were calculated according to the prices in the Egyptian market during the study period. The economic evaluation was calculated by the following equation:

$$\text{Net income (LE)} = \text{Total fish price (LE)} - \text{Total costs (LE)}$$

#### 7. Statistical analysis

Statistical analysis and graphics of data were conducted by using Microsoft Excel and Minitab software, Ver. 5.2 under windows programs. Data were analyzed by using the All Pairwise Multiple Comparison Procedures via Holm-Sidak Method (Holm, 1979) at 95% confidence.

## RESULTS

### 1. Fish growth parameters

The effect of fish overwintering in earthen aquaculture (**EP<sub>1</sub>**, **EP<sub>2</sub>** and **EP<sub>3</sub>**) and underground water RAS aquaculture (**CP<sub>1</sub>**, **CP<sub>2</sub>**, **CP<sub>3</sub>**, **CP<sub>4</sub>** and **CP<sub>5</sub>**) on growth in length (cm and %): length gain (LG cm), daily length gain (DLG mm/fish/day); growth in weight (g and %): total weight gain (WG g), daily gain (DWG g/fish/day) and specific growth rate (SGR %/day); feed intake (FI g/fish) of *Oreochromis niloticus* are given in Tables (1- 4) and are graphically presented in Figs. (1, 2).

#### 1.1. Growth in length (L, cm & %)

Data in Tables (1, 2) and Fig. (1) show that the Nile tilapia fingerlings reared in earthen ponds and RAS aquacultures exhibited great variations in body length. The highest average body length (**9.64 ± 0.539** cm) was recorded in earthen ponds, and the lowest average body length (**7.50 ± 0.470** cm) was recorded in the underground water RAS aquaculture. While, the highest average growth in length of **143.27 ± 16.959%** was recorded in RAS treatment, and the lowest average growth in length (**96.44 ± 13.492** %) was registered in the control earthen ponds.

### 1.2. Length gain (LG, cm/fish) and average daily length gain (DLG, $\mu$ /fish/day)

Results showed that the Nile tilapia fingerlings reared in earthen ponds and RAS aquacultures exhibited convergent variations in length gain and average length gain. The highest length gain ( $4.48 \pm 0.539$  cm/fish) was recorded in earthen ponds, and the lowest length gain ( $4.30 \pm 0.442$  cm/fish) was recorded in fishes reared in underground water RAS aquaculture (Table 2 & Fig. 1). The highest daily length gain ( $400.14 \pm 4.791 \mu$ /fish/day) was recorded for fishes reared in earthen ponds. While, the highest length gain ( $384.21 \pm 39.468 \mu$ /fish/day) was recorded for fingerlings reared in RAS aquaculture (Table 2 & Fig. 1).

### 1.3. Growth in body weight (W, g & %)

Tables (3, 4) and Fig. (2) show that, *Oreochromis niloticus* fingerlings reared in different earthen ponds and underground water RAS aquaculture ponds exhibited great variations in body weight. The highest average body weight ( $14.74 \pm 4.765$  g) was recorded in earthen ponds, while the lowest average body weight ( $10.95 \pm 2.356$  g) was recorded in underground water RAS aquaculture. The highest growth in weight ( $260 \pm 96.8$  %) was recorded for the underground water RAS aquaculture, while the lowest growth in weight ( $258 \pm 45.5$  %) was registered for the earthen ponds.

### 1.4. Total weight gain (WG, g/fish) and average daily weight gain (DWG, mg/fish/day)

The highest total weight gain ( $10.26 \pm 3.566$  g) was recorded for fish in the control earthen ponds, and the lowest total weight gain ( $6.89 \pm 2.538$  g) was recorded for fish reared in underground water RAS aquaculture ponds (Table 4 & Fig. 2). Moreover, the highest average daily weight gain ( $91.58 \pm 31.837$  mg/fish/day) was recorded in the earthen ponds, while the lowest average daily weight gain ( $61.51 \pm 22.659$  mg/fish/day) was recorded in the underground water RAS aquaculture ponds (Table 4 & Fig. 2).

### 1.5. Specific growth rate (% / day)

The results showed that the highest average specific growth rate ( $1.36 \pm 0.121$  %) was recorded in the earthen ponds. While, the lowest average specific growth rate ( $1.13 \pm 0.316$  %) was detected in the underground water RAS aquaculture ponds (Table 4).

### 1.6. Statistical analysis of growth performance

The results showed that the final length and growth in length were significantly varied between fish reared in the earthen ponds and those reared in RAS treatment concrete ponds. On the other hand, the growth performance parameters in length ( $L_f$ , LG, DLG and G in L) recorded a non-significant variation ( $P > 0.05$ ) between all RAS ponds, and the same manner was shown between all earthen ponds.

The results showed that the growth performance parameters in weight ( $W_f$ , WG, DWG, G in W and SGR) were significantly varied between all concrete ponds of the recycle aquaculture system (RAS) treatment and that of the control ponds.

The growth in weight (GW) was significantly varied ( $P < 0.05$ ) between CP<sub>1</sub>, CP<sub>2</sub> and CP<sub>5</sub>. On other hand, no significant differentiation ( $P > 0.05$ ) was detected in GW between CP<sub>3</sub> and CP<sub>4</sub>, and the same manner was recorded in the earthen ponds (EP<sub>1</sub>, EP<sub>2</sub> and EP<sub>3</sub>). The final weight ( $W_f$ ) was significantly varied ( $P < 0.05$ ) for EP<sub>3</sub> in comparison to other ponds and tanks. Additionally, the weight gain (WG) and daily weight gain (DWG) were significantly varied ( $P < 0.05$ ) for EP<sub>3</sub>, compared to other ponds and tanks. The specific growth rate was significantly varied ( $P < 0.05$ ) between EP<sub>2</sub>, compared to EP<sub>1</sub> and EP<sub>3</sub>, except between tank 3 and 4, no significant differentiation ( $P > 0.05$ ) was detected.

**Table 1.** Total fish length in cm (average  $\pm$  SD) of *O. niloticus* for control and recycle aquaculture system (RAS) treatment during winter months

Treatment		Months			
		December (Zero-day)	January	February	March
Control	EP <sub>1</sub>	4.48 $\pm$ 1.062	5.71 $\pm$ 0.669	7.43 $\pm$ 0.659	9.02 $\pm$ 1.073
	EP <sub>2</sub>	5.52 $\pm$ 1.23	6.81 $\pm$ 0.75	8.45 $\pm$ 1.78	9.95 $\pm$ 0.89
	EP <sub>3</sub>	5.48 $\pm$ 1.01	6.27 $\pm$ 0.73	7.80 $\pm$ 0.64	9.95 $\pm$ 0.82
<b>Total</b>		<b>5.16<math>\pm</math> 0.589</b>	<b>6.26<math>\pm</math> 0.72</b>	<b>7.89<math>\pm</math> 1.03</b>	<b>9.64<math>\pm</math> 0.539</b>
RAS treatment	CP <sub>1</sub>	3.03 $\pm$ 0.551	3.84 $\pm$ 0.689	4.61 $\pm$ 0.539	6.87 $\pm$ 1.013
	CP <sub>2</sub>	3.20 $\pm$ 0.50	4.0 $\pm$ 0.62	5.07 $\pm$ 1.14	7.13 $\pm$ 2.24
	CP <sub>3</sub>	3.52 $\pm$ 0.73	4.97 $\pm$ 0.83	6.45 $\pm$ 0.93	7.89 $\pm$ 1.27
	CP <sub>4</sub>	3.20 $\pm$ 0.55	4.95 $\pm$ 1.01	6.45 $\pm$ 1.86	7.73 $\pm$ 1.44
	CP <sub>5</sub>	3.0 $\pm$ 0.54	4.04 $\pm$ 0.50	5.34 $\pm$ 0.78	7.88 $\pm$ 1.25
<b>Total</b>		<b>3.26<math>\pm</math>0.228</b>	<b>4.36<math>\pm</math>0.73</b>	<b>5.58<math>\pm</math>1.05</b>	<b>7.30<math>\pm</math>0.532</b>

**Table 2.** Growth performance parameters in length (average $\pm$  SD) of *O. niloticus* for control and recycle aquaculture system (RAS) treatment during winter months

Treatment		Initial length (g)	Final length (g)	Length gain (cm/fish)	Daily length gain ( $\mu$ /fish/day)	Growth in length (%)
Control	EP <sub>1</sub>	4.48 $\pm$ 1.062	9.02 $\pm$ 1.073	4.45 <sup>a</sup> $\pm$ 1.448	405.3 <sup>a</sup> $\pm$ 129.26	111.98 <sup>c</sup> $\pm$ 53.58
	EP <sub>2</sub>	5.52 $\pm$ 1.23	9.95 $\pm$ 0.89	4.43 <sup>ab</sup> $\pm$ 1.550	395.83 <sup>ab</sup> $\pm$ 138.44	89.62 <sup>d</sup> $\pm$ 46.217
	EP <sub>3</sub>	5.48 $\pm$ 1.01	9.95 $\pm$ 0.82	4.47 <sup>ab</sup> $\pm$ 1.18	399.29 <sup>ab</sup> $\pm$ 105.47	87.72 <sup>d</sup> $\pm$ 36.810
<b>Total</b>		<b>5.16<math>\pm</math> 0.589</b>	<b>9.64<math>\pm</math> 0.539</b>	<b>4.48<math>\pm</math> 0.539</b>	<b>400.14<math>\pm</math> 4.791</b>	<b>96.44<math>\pm</math> 13.492</b>
RAS treatment	CP <sub>1</sub>	3.08 $\pm$ 0.551	6.87 $\pm$ 1.013	3.80 <sup>b</sup> $\pm$ 1.027	338.88 <sup>b</sup> $\pm$ 91.73	127.84 <sup>bc</sup> $\pm$ 41.72
	CP <sub>2</sub>	3.20 $\pm$ 0.50	7.13 $\pm$ 2.24	3.93 <sup>b</sup> $\pm$ 2.473	351.07 <sup>b</sup> $\pm$ 220.80	132.38 <sup>bc</sup> $\pm$ 102.88
	CP <sub>3</sub>	3.52 $\pm$ 0.73	7.89 $\pm$ 1.27	4.38 <sup>ab</sup> $\pm$ 1.549	390.92 <sup>ab</sup> $\pm$ 138.28	135.88 <sup>b</sup> $\pm$ 69.39
	CP <sub>4</sub>	3.20 $\pm$ 0.55	7.73 $\pm$ 1.44	4.54 <sup>ab</sup> $\pm$ 1.663	404.96 <sup>ab</sup> $\pm$ 148.48	150.91 <sup>ab</sup> $\pm$ 73.182
	CP <sub>5</sub>	3.0 $\pm$ 0.54	7.88 $\pm$ 1.25	4.87 <sup>a</sup> $\pm$ 1.243	435.22 <sup>a</sup> $\pm$ 110.90	169.36 <sup>a</sup> $\pm$ 58.637
<b>Total</b>		<b>3.26<math>\pm</math>0.196</b>	<b>7.50<math>\pm</math>0.470</b>	<b>4.30<math>\pm</math>0.442</b>	<b>384.21<math>\pm</math>39.468</b>	<b>143.27<math>\pm</math>16.959</b>

\*means significantly varied ( $P<0.05$ ); different letters in the same column are significantly varied ( $P<0.05$ ).

**Table 3.** Growth in weight in grams (average  $\pm$  SD) of *O. niloticus* for control and recycle aquaculture system (RAS) treatment during winter months

Treatment		Months			
		December (Zero-day)	January	February	March
Control	EP <sub>1</sub>	2.86 $\pm$ 0.822	4.83 $\pm$ 0.799	7.57 $\pm$ 1.378	10.10 $\pm$ 2.244
	EP <sub>2</sub>	5.17 $\pm$ 1.49	6.31 $\pm$ 1.34	11.84 $\pm$ 3.21	14.52 $\pm$ 2.01
	EP <sub>3</sub>	5.43 $\pm$ 1.42	6.83 $\pm$ 1.38	14.98 $\pm$ 3.22	19.62 $\pm$ 4.30
<b>Total</b>		<b>4.49<math>\pm</math> 1.413</b>	<b>5.99<math>\pm</math> 1.173</b>	<b>11.463<math>\pm</math> 2.603</b>	<b>14.74<math>\pm</math> 4.765</b>
RAS treatment	CP <sub>1</sub>	3.88 $\pm$ 2.687	4.38 $\pm$ 2.320	4.68 $\pm$ 1.733	8.37 $\pm$ 3.3489
	CP <sub>2</sub>	4.59 $\pm$ 2.31	5.53 $\pm$ 2.78	6.76 $\pm$ 4.51	8.80 $\pm$ 7.46
	CP <sub>3</sub>	4.0 $\pm$ 2.41	6.46 $\pm$ 3.24	8.95 $\pm$ 5.17	11.50 $\pm$ 4.64
	CP <sub>4</sub>	4.06 $\pm$ 2.40	7.28 $\pm$ 4.15	10.70 $\pm$ 1.86	12.08 $\pm$ 6.99
	CP <sub>5</sub>	3.78 $\pm$ 1.75	5.53 $\pm$ 1.72	8.30 $\pm$ 3.19	14.02 $\pm$ 6.96
<b>Total</b>		<b>4.16<math>\pm</math>0.377</b>	<b>5.84<math>\pm</math>2.84</b>	<b>7.88<math>\pm</math>3.29</b>	<b>10.95<math>\pm</math>2.356</b>

**Table 4.** Growth performance parameters in weight (average  $\pm$  SD) of *O. niloticus* for control and recycle aquaculture system (RAS) treatment during winter months

Treatment		Initial weight (g)	Final weight (g)	Weight gain (g/fish)	Daily weight gain (mg/fish/day)	Growth in weight (%)	Specific growth rate (%/day)
Control	EP <sub>1</sub>	2.86 $\pm$ 0.822	10.10 <sup>c</sup> $\pm$ 2.244	7.23 <sup>c</sup> $\pm$ 2.441	64.57 <sup>c</sup> $\pm$ 21.794	279 <sup>ab</sup> $\pm$ 126	1.38 <sup>ab</sup> $\pm$ 0.34
	EP <sub>2</sub>	5.17 $\pm$ 1.49	14.52 <sup>b</sup> $\pm$ 2.01	9.35 <sup>b</sup> $\pm$ 2.679	83.49 <sup>b</sup> $\pm$ 23.919	206 <sup>c</sup> $\pm$ 981	1.24 <sup>c</sup> $\pm$ 0.31
	EP <sub>3</sub>	5.43 $\pm$ 1.42	19.62 <sup>a</sup> $\pm$ 4.30	14.19 <sup>a</sup> $\pm$ 4.648	126.68 <sup>a</sup> $\pm$ 41.497	289 <sup>a</sup> $\pm$ 136	1.47 <sup>a</sup> $\pm$ 0.34
<b>Total</b>		<b>4.49<math>\pm</math> 1.413</b>	<b>14.74<math>\pm</math>4.765</b>	<b>10.26<math>\pm</math> 3.566</b>	<b>91.58<math>\pm</math> 31.837</b>	<b>258 <math>\pm</math> 45.5</b>	<b>1.36<math>\pm</math> 0.121</b>
RAS treatment	CP <sub>1</sub>	3.88 $\pm$ 2.687	8.37 <sup>c</sup> $\pm$ 3.3489	4.49 <sup>de</sup> $\pm$ 3.951	40.07 <sup>de</sup> $\pm$ 35.281	172 <sup>d</sup> $\pm$ 146	0.97 <sup>d</sup> $\pm$ 0.56
	CP <sub>2</sub>	4.59 $\pm$ 2.31	8.80 <sup>c</sup> $\pm$ 7.46	4.21 <sup>c</sup> $\pm$ 8.033	37.60 <sup>e</sup> $\pm$ 71.720	138 <sup>e</sup> $\pm$ 228	0.66 <sup>e</sup> $\pm$ 0.86
	CP <sub>3</sub>	4.0 $\pm$ 2.14	11.50 <sup>bc</sup> $\pm$ 4.64	7.50 <sup>bcd</sup> $\pm$ 5.498	66.94 <sup>bcd</sup> $\pm$ 49.090	331 <sup>ab</sup> $\pm$ 377	1.28 <sup>bc</sup> $\pm$ 0.74
	CP <sub>4</sub>	4.06 $\pm$ 2.40	12.08 <sup>bc</sup> $\pm$ 6.99	8.02 <sup>bc</sup> $\pm$ 7.356	71.58 <sup>bc</sup> $\pm$ 65.680	330 <sup>b</sup> $\pm$ 421	1.26 <sup>bc</sup> $\pm$ 0.77
	CP <sub>5</sub>	3.78 $\pm$ 1.75	14.01 <sup>b</sup> $\pm$ 6.22	10.23 <sup>b</sup> $\pm$ 6.016	91.37 <sup>b</sup> $\pm$ 53.715	331 <sup>a</sup> $\pm$ 228	1.47 <sup>a</sup> $\pm$ 0.48
<b>Total</b>		<b>4.06<math>\pm</math>0.313</b>	<b>10.95<math>\pm</math>2.356*</b>	<b>6.89<math>\pm</math>2.538*</b>	<b>61.51<math>\pm</math>22.659*</b>	<b>260<math>\pm</math>96.8*</b>	<b>1.13<math>\pm</math>0.316*</b>

\*means significantly varied ( $P < 0.05$ ); different letters in the same column are significantly varied ( $P < 0.05$ ).

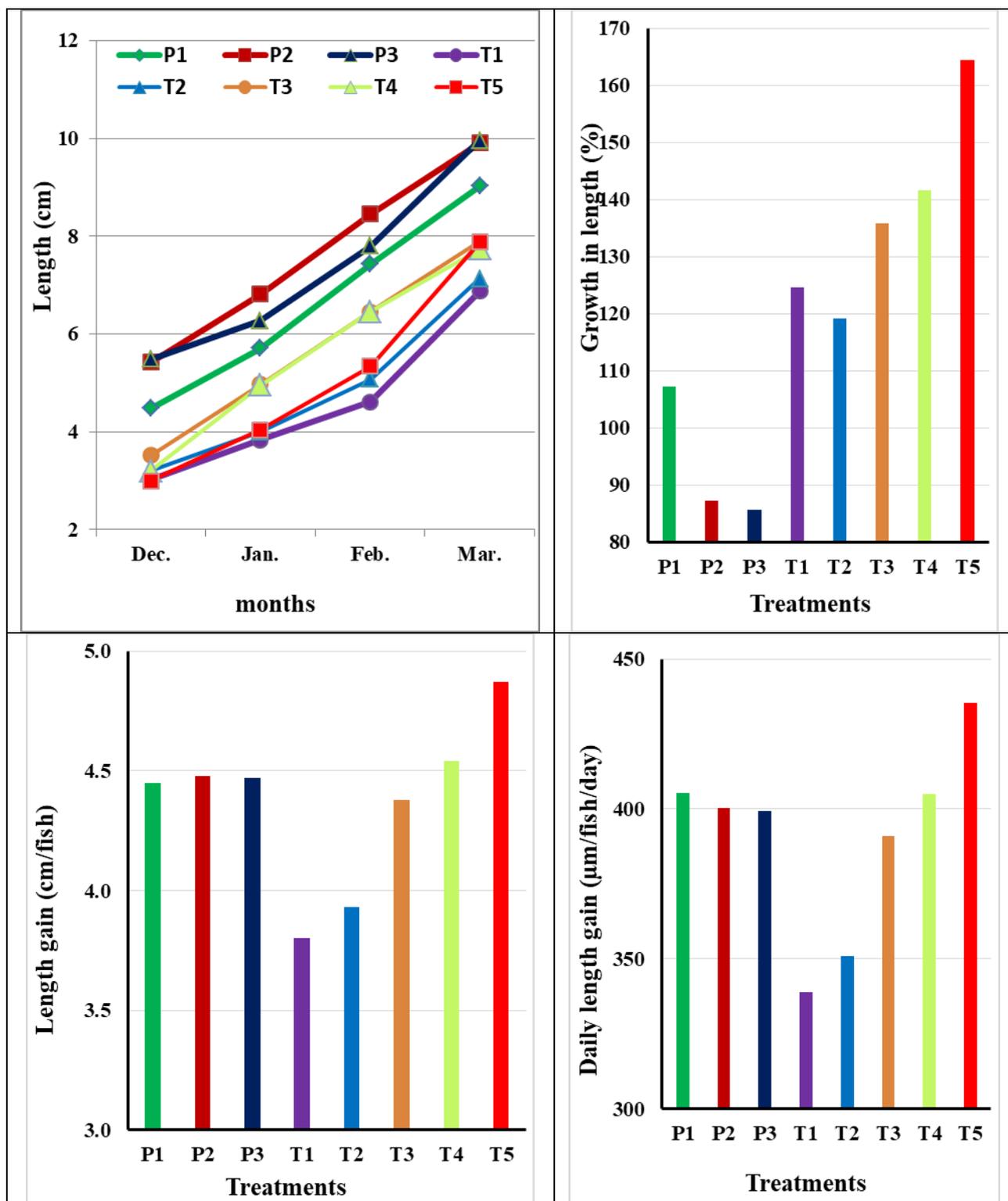


Fig. 1. Effect of *O. niloticus* fingerlings overwintering in control earthen ponds (P1, P2 and P3) and recycle aquaculture system (RAS) ponds (T1, T2, T3, T4 and T5) on length growth parameters

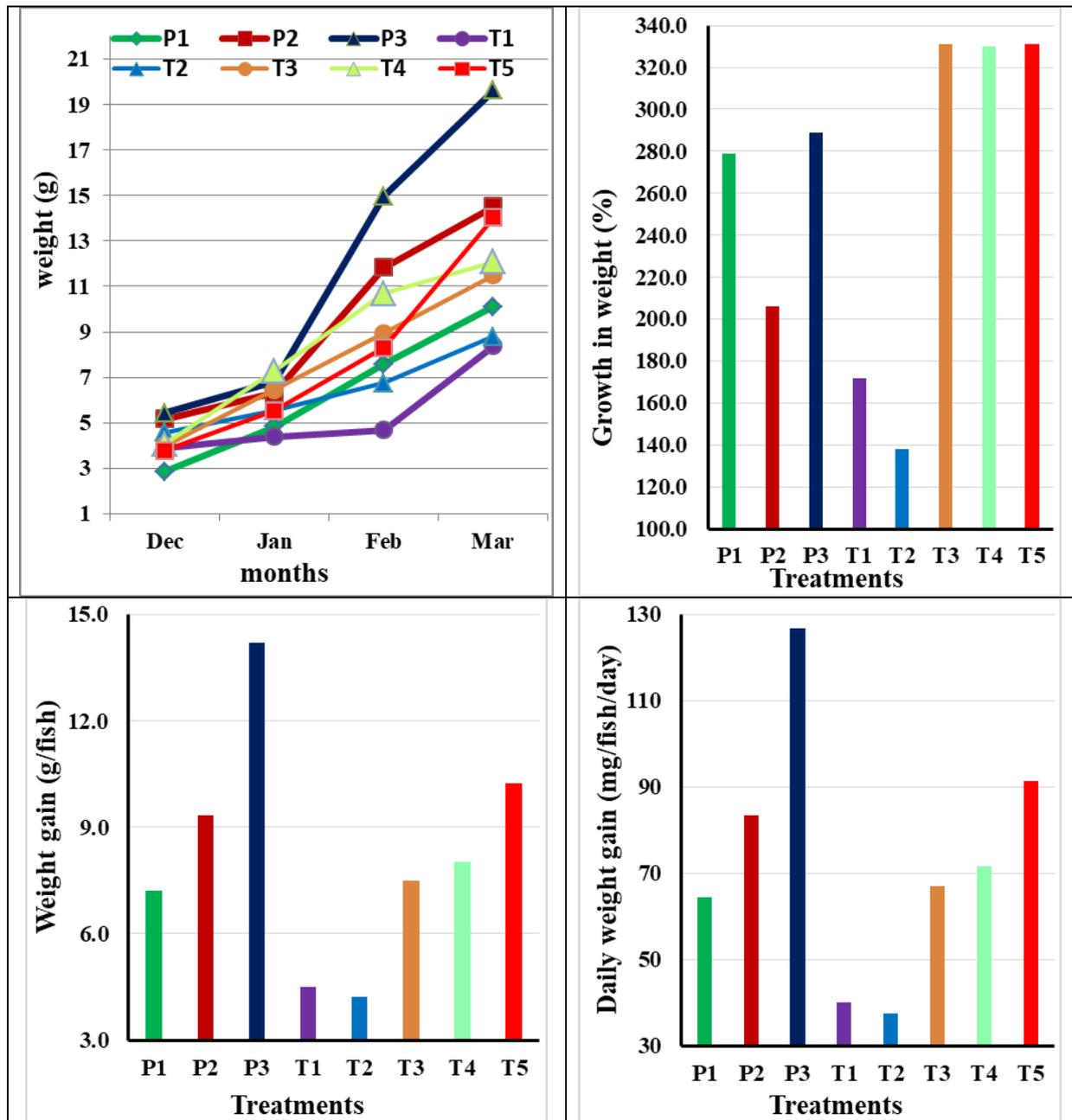


Fig. 2. Effect of *O. niloticus* fingerlings overwintering in control earthen ponds (P1, P2 and P3) and recycle aquaculture system (RAS) ponds (T<sub>1</sub>, T<sub>2</sub> T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>) on weight growth parameters

### 3. Feed utilization parameters

#### 3.1. Total feed intake (g/fish)

Results in Table (5) show that, *O. niloticus* fingerlings reared in different earthen ponds and underground water RAS aquaculture exhibited great variations in feed intake. The highest average feed intake ( $43.40 \pm 7.910$  g) was recorded in the earthen ponds. Whereas, the lowest average feed intake ( $37.86 \pm 2.106$  g) was in the underground water RAS aquaculture.

#### 3.2. Food conversion ratio and feed efficiency ratio (FER)

Results showed that the best food conversion ratio ( $0.2 \pm 0.050$ ) was recorded for fish fingerlings of the Nile tilapia reared in the underground water RAS ponds; while, the worst food conversion ratio ( $0.59 \pm 0.409$ ) was recorded for fish reared in the earthen ponds (Table

5). While, the highest feed efficiency ratio ( $5.7 \pm 1.482$ ) was recorded in underground water RAS ponds, and the lowest feed efficiency ratio ( $2.32 \pm 1.435$ ) was recorded in earthen ponds (Table 5).

### 3.3. Protein intake (PI / g) and protein efficiency ratio (PER)

Results showed that the highest protein intake ( $2.32 \pm 1.435$  g) was recorded in the earthen ponds, and the lowest protein intake ( $0.6 \pm 0.031$  g) was recorded for underground water RAS aquaculture (Table 5). While, the highest protein efficiency ratio ( $12.0 \pm 4.913$ ) was recorded in the underground water RAS aquaculture, and the lowest protein efficiency ratio ( $5.38 \pm 3.514$ ) was recorded for earthen ponds (Table 5).

**Table 5.** Feed utilization parameters of *O. niloticus* for control and recycle aquaculture system (RAS) treatment during winter months

Treatment		Feed intake (g/fish)	Food conversion ratio	Feed efficiency ratio	Protein intake (g/fish)	Protein efficiency ratio
Control	EP <sub>1</sub>	10.588	1.049	0.953	3.176	2.277
	EP <sub>2</sub>	6.667	0.459	2.178	2.000	4.675
	EP <sub>3</sub>	5.143	0.262	3.814	1.543	9.196
<b>Total</b>		<b>7.47 ± 2.809</b>	<b>0.59 ± 0.409</b>	<b>2.32 ± 1.435</b>	<b>2.24 ± 0.843</b>	<b>5.38 ± 3.514</b>
RAS Treatment	CP <sub>1</sub>	2.107	0.252	3.973	0.632	7.099
	CP <sub>2</sub>	1.985	0.226	4.432	0.595	7.073
	CP <sub>3</sub>	1.934	0.168	5.943	0.580	12.920
	CP <sub>4</sub>	1.895	0.157	6.373	0.568	14.105
	CP <sub>5</sub>	1.838	0.131	7.621	0.551	18.556
<b>Total</b>		<b>2.0* ± 0.102</b>	<b>0.2* ± 0.050</b>	<b>5.7* ± 1.482</b>	<b>0.6* ± 0.031</b>	<b>12.0* ± 4.913</b>

\*means significantly varied ( $P < 0.05$ ).

### 4. Mortality rate (MR)

Results in Table (6) reveals that, the lowest mortality rate ( $9.1 \pm 5.09\%$ ) was recorded in underground water RAS ponds, and the highest mortality rate ( $80.33 \pm 6.429\%$ ) was recorded for earthen ponds.

**Table 6.** Mortality rate of *O. niloticus* for fish reared in control earthen ponds and recycle aquaculture system (RAS) ponds during winter months

Treatment		Initial number	Mortality rate	
			Dead fish	%
Control	EP <sub>1</sub>	350000	290500	83
	EP <sub>2</sub>	300000	219000	73
	EP <sub>3</sub>	250000	212500	85
<b>Total</b>		<b>900000</b>	<b>272000</b>	<b>80.33* ± 6.429</b>
RAS Treatment	CP <sub>1</sub>	48000	7000	14.58
	CP <sub>2</sub>	42000	5000	11.91
	CP <sub>3</sub>	36000	2500	6.94
	CP <sub>4</sub>	30000	1500	5.0
	CP <sub>5</sub>	24000	500	2.08
<b>Total</b>		<b>180000</b>	<b>16500</b>	<b>9.1* ± 5.09</b>

\*means significantly varied ( $P < 0.05$ ).

## 5. Economical evaluation

The economic evaluation to the experiment during winter 2019- 2020 indicated that, the total feed intake of the Nile tilapia *Oreochromis niloticus* recorded for earthen ponds (EP<sub>1</sub>, EP<sub>2</sub> and EP<sub>3</sub>) was 1130kg. The total feed intake used in RAS aquaculture ponds (CP<sub>1</sub>, CP<sub>2</sub>, CP<sub>3</sub>, CP<sub>4</sub> and CP<sub>5</sub>) was 102kg (Table 7).

It was showed that the total costs including feed cost, fish fry cost and operation costs were 51300, 45400 and 39500 LE for earthen ponds EP<sub>1</sub>, EP<sub>2</sub> and EP<sub>3</sub>, respectively. In case of RAS aquaculture experiment, the total costs were 16944, 149954, 13008, 11040 and 9072 L.E for concrete ponds from CP1 to CP5, respectively (Table 7).

The price of produced fingerlings was estimated according to their size and weight. Thus, the highest income for fish price was recorded for fish produced in earthen pond EP<sub>3</sub>. Therefore, the net income showed that the maximum value of positive net income was recorded (307.4 LE/1000 fingerlings) in RAS aquaculture CP<sub>5</sub>, followed by CP<sub>4</sub> (249.5 LE/1000 fingerlings) and CP<sub>3</sub> (243.5 LE/1000 fingerlings). While, in earthen ponds, a negative net income was recorded only at EP<sub>1</sub> (-61.57 LE/1000 fingerlings). The other earthen ponds recorded positive net income, 139.5 and 37.7 LE/1000 fingerlings for EP<sub>3</sub> and EP<sub>2</sub> respectively (Table 7).

**Table 7.** The economic evaluation of *O. niloticus* production for earthen ponds and RAS treatments during winter months 2019-2020

Subject		Unit price (LE)	Control			RAS treatment				
			EP 1	EP 2	EP 3	CP 1	CP 2	CP 3	CP 4	CP 5
Costs	Feed intake (Kg)		630	540	450	86.4	73.4	64.8	54	43.2
	Feed cost (LE)	10	6300	5400	4500	864	734	648	540	432
	Fingerlings (1000)		350	300	250	48	42	36	30	24
	Fish cost (LE)	100	35000	30000	25000					
		300				14880	13020	11160	9300	7440
	Operation cost (LE) *		10000	10000	10000	1200	1200	1200	1200	1200
Total costs (LE)		<b>51300</b>	<b>45400</b>	<b>39500</b>	<b>16944</b>	<b>14954</b>	<b>13008</b>	<b>11040</b>	<b>9072</b>	
Income	Final fish no. (1000 fish)		59.5	81.0	87.5	41	37	33.5	28.5	23.5
	Total income (LE/1000 fish)	500-700-850	29750	56700	74375					
		450 – 650-700				18450	16650	21775	18525	16450
Net income (LE)	Whole	Total	-21550	11300	34875	1506	1696	8767	7485	7378
		Average	24625			5366.4				
	Per 1000 fingerlings	Total	-61.57	37.7	139.5	31.38	40.38	243.53	249.50	307.42
		Average	38.54			174.4				

\*Operation costs include workers, electricity, service...etc.

## DISCUSSION

The optimum temperature for the growth of most tilapia species is between 25 and 28 degrees Celsius. Their reproduction stops at 22°C and feeds below 20°C. Cichlids cannot tolerate temperatures below 10-12°C for more than a few days. The inability of tilapia to tolerate low winter temperatures in temperate and some subtropical regions, such as in Egypt, is a major economic concern because it causes poor growth and mass mortality during the winter (Costa-Pierce, 2003; Charo-Karisa *et al.*, 2005; El-Sayed, 2006; Ernst *et al.*, 2007).

The present results showed that the mortality rate of Nile tilapia fingerlings reared in the earthen ponds was very high ( $80.33 \pm 6.429\%$ ) where the air temperature was reached to the very cold degrees at the region of the farm during the winter time (**Table, 8**). This result is consistent with most previously studied, where the temperature fluctuated from 6 to 14 °C at night during January and February 2020 and this resulted in a high mortality rate. On the other hand, the mortality rate in RAS concrete ponds was very low ( $9.1 \pm 5.09$ ), where the temperature in the farm was adjusted to  $28 \pm 1$  °C.

The temperature is one of the most important factors affecting the growth, survival, physiological, biochemical and immunological functions of fish. At lower temperatures, three basic changes occur in fish. First, the cold temperature activates the neuroendocrine pathway causing the release of cortisol, thyroxine, or catecholamines; Subsequently, changes occur in the regulatory functions, hematologic and enzymatic functions of the blood, and metabolism; Eventually, abnormal fish behavior appears, growth stops, and chances of infection and mortality increase (**Chen *et al.*, 2002; Moreira *et al.*, 2007; Ndong *et al.*, 2007; Chebaani *et al.*, 2014; Chebaani *et al.*, 2014; Qiang *et al.*, 2014; Ma *et al.*, 2015; Younis, 2015**).

**Table (8): Variation in air temperatures °C during winter months 2019-2020 (After: Weather and Climate, 2023)**

Months	Air temperatures (°C)			
	Minimum		Maximum	
	Range	Average $\pm$ SD	Range	Average $\pm$ SD
December 2019	07 - 16	13.00 $\pm$ 1.28	16 - 24	20.00 $\pm$ 2.4
January 2020	06 - 14	11.00 $\pm$ 2.15	12 - 24	19.00 $\pm$ 2.65
February 2020	06 - 14	12.00 $\pm$ 1.99	13 - 25	20.00 $\pm$ 2.57
March 2020	06 - 16	14.00 $\pm$ 2.68	16 - 31	26.00 $\pm$ 3.45
April 2020	11 - 19	17.00 $\pm$ 3.04	18 - 36	29.63 $\pm$ 4.27

The results showed that the fingerlings of Nile tilapia reared in earthen ponds exhibited highest averages of most growth performance parameters (growth in length, length gain, daily length gain, growth in weight, total weight gain, daily weight gain, specific growth rate). But, the fingerlings reared in RAS ponds exhibited the highest averages of growth in weight with the decrease of fish stocking density in CP<sub>3</sub>, CP<sub>4</sub> and CP<sub>5</sub>. This results agreed with that obtained by many authors (**Abd El-aal, 2008; Azab *et al.*, 2018**).

Also, the present study showed that the Nile tilapia fingerling reared RAS concrete ponds exhibited the best averages of feed utilization parameters (feed intake, feed conversion ratio, protein efficiency ratio and feed efficiency). These results are in agreement with that obtained by **Abd El-aal (2008)**. His results showed that feed utilization parameters were significantly affected by pond depth and pond covering by polyethylene sheets. He recommended using pond covering 100% polyethylene sheet, which better way to overwintering fish reared in concrete pond.

The present results of economic evaluation showed that fish stocking density during overwintering affect on the net income either in earthen ponds or in RAS concrete ponds.

## REFERENCES

- Abdel-Aal, M.M. (2008).** Effects of over-wintering and water depth on growth performance of Nile tilapia (*Oreochromis niloticus*). In: Proceedings of the 8<sup>th</sup> International Symposium on Tilapia in Aquaculture. Cairo: The Central Laboratory for Aquaculture Research, Ministry of Agriculture and Land Reclamation, pp. 297–306.
- Abdel-Ghany, H.M.; El-Sayed, A.F.M.; Ezzat, A.A.; Essa, M.A. and Helal, A.M. (2019).** Dietary lipid sources affect cold tolerance of Nile tilapia (*Oreochromis niloticus*). *J. Therm. Biol.*, **79**: 50–55. <https://doi.org/10.1016/j.jtherbio.2018.11.009>.
- Abdel-Hakim, N.F.; Lashin, M.E.; Alazab, A.A.; Radwan, I.A. and Abdelhamid, A.F.B. (2016).** Effect of dietary crude protein level and stocking rate of mono sex all male Nile Tilapia reared under Recirculating Aquaculture System., *J. Animal and Poultry Prod.*, Mansoura Univ., **7**(1): 1 – 13.
- Azab, A.M.; Khalaf-Allah, H.M.M.; Khattaby, A.A.; Sadek, A.S.M. and Abdel-Ghany, M.O.M. (2018).** Effect of stocking density and feeding rate on growth performance and total production of Nile Tilapia, *Oreochromis niloticus* reared in earthen ponds. *Egyptian Journal for Aquaculture*, **8**(3): 33-54.
- Azaza, M.S.; Dhraïef, M.N. and Kraïem, M.M. (2008).** Effects of water temperature on growth and sex ratio of juvenile Nile tilapia, *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia. *J. Therm. Biol.*, **33**: 98–105.
- Castell, J.D. and Tiews, K. (1980):** Report of the EIFAC, IUNS and ICES Working Group on the Standardization of Methodology in Fish Research. Hamburg, FRG, Germany, IFAC Tech., **3**: 24 pp.
- Chang, Y.M.; Cao, D.C.; Sun, X. and Liang, L.Q. (2006).** Changes of serum biochemical indices of common carp affected by cold temperatures. *Chin. J. Fish.*, **2**: 71-75.
- Charo-Karisa, H.; Rezk, M.A.; Bovenhuis, H. and Komen, H. (2005).** Heritability of cold tolerance in Nile tilapia, *Oreochromis niloticus*, juveniles. *Aquaculture*, **249**: 115–123.
- Chebaani, N.; Guardiola, F.A.; Sihem, M.; Nabil A, Oumouna, M.; Meseguer, J.; Esteban, M.A. and Cuesta, A. (2014).** Innate humoral immune parameters in *Tilapia zillii* under acute stress by low temperature and crowding. *Fish Physiol. Biochem.*, **40**: 797–804.
- Chen, W.H.; Sun, L.T.; Tsai, C.L.; Song, Y.L. and Chang, C.F. (2002):** Cold-stress induced the modulation of catecholamines, cortisol, immunoglobulin M, and leukocyte haptocytosis in tilapia. *Gen Comp Endocrinol* 126-90:100.
- Corrêa, C.F.; Nobrega, R.O.; Block, J.M. and Fracalossi, D.M. (2018).** Mixes of plant oils as fish oil substitutes for Nile tilapia at optimal and cold suboptimal temperature. *Aquaculture*, **497**: 82–90.
- Corrêa, C.F.; Nobrega, R.O.; Mattioni, B.; Block, J.M. and Fracalossi, D.M. (2017):** Dietary lipid sources affect the performance of Nile tilapia at optimal and cold, suboptimal temperatures. *Aquacult. Nutr.*, **23**: 1016–1026.
- Costa-Pierce, B.A. (2003).** Rapid evolution of an established feral tilapia (*Oreochromis* spp.): the need to incorporate invasion science into regulatory structures. *Biol. Invasions*, **5** (1–2): 71–84.

- Dan, N.C. and Little, D.C. (2000).** Overwintering performance of Nile tilapia *Oreochromis niloticus* (L.) broodfish and seed at ambient temperatures in northern Vietnam. *Aquaculture Research*, **31**: 485–493.
- Egna, H.S. and Boyd, C.E. (2017).** Dynamics of Pond Aquaculture. CRC Press, p. 472.
- El-Sayed, A. F. M. (2006).** Tilapia Culture. CABI, Cambridge, MA, USA.
- El-Sayed, A.F.M.; El-Ghoboshi, A. and Al-Amoudi, M. (1996).** Effects of pond depth and water temperature on growth, mortality and body composition of Nile tilapia (*Oreochromis niloticus*). *J. Aquac. Res. Dev.*, **27**: 681–687.
- Ernst, D.H.; Watanabe, W.O.; Ellingson, L.J.; Wicklund, R.I. and Olla, B.L. (2007).** Commercial-scale production of Florida red tilapia seed in low- and brackish-salinity tanks. *J. World Aquacult. Soc.*, **22**: 36–44.
- FAO “Food and Agriculture Organization of the United Nations” (2020).** Global Aquaculture Production 1950-2018. <http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en>.
- Holm Sture, (1979).** A simple sequentially rejective multiple test procedure, *Scandinavian Journal of Statistics*, **6** (2): 65-70.
- Jobling, M., (1994).** Fish Bioenergetics-Fish and Fisheries Series, Vol. 13. Chapman & Hall, London, UK.
- Lin, Z. (1991):** Pond Fisheries in China. International Academic Publishers.
- Ma, X.Y.; Qiang, J.; He, J.; Gabriel, N.N. and Xu, P. (2015).** Changes in the physiological parameters, fatty acid metabolism, and SCD activity and expression in juvenile GIFT tilapia (*Oreochromis niloticus*) reared at three different temperatures. *Fish Physiol. Biochem.*, **41**: 937–950. <https://doi.org/10.1007/s10695-015-0059-4>.
- Moreira I.S.; Peres. H.; Couto, A.; Enes, P. and Oliva-Teles, A. (2007):** Temperature and dietary carbohydrate level effects on performance and metabolic utilisation of diets in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, **74**: 153–160.
- Ndong, D.; Chen, Y.; Lin, Y.; Vaseeharan, B. and Chen, J. (2007):** The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus iniae* under stress in low and high temperatures. *Fish Shellfish Immunol.*, **22**: 686–694.
- Nobrega, R.O.; Batista, R.O.; Corrêa, C.F.; Mattioni, B.; Filer, K.; Pettigrew, J.E. and Fracalossi, D.M. (2019).** Dietary supplementation of *Aurantiochytrium* sp. meal, a docosahexaenoic acid source, promotes growth of Nile tilapia at a suboptimal low temperature. *Aquaculture*, **507**: 500–509. <https://doi.org/10.1016/j.aquaculture>. 2019. 04. 030
- Qiang, J.; He, J.; Xu, P.; Kpundeh, M. and Zhu, Z. (2014).** Optimization of culture conditions for larval GIFT tilapia *Oreochromis niloticus* using response surface methodology and effects of HAMP-1 and c-type lysozyme mRNA expression in liver. *Aquacult. Int.*, **22**: 975–991.
- Recker, W.E. (1975).** Computation and interpretation of biological statistics of fish populations. *Fish. Res. Board Can. Bull.*, **191**: 1-382.
- Shi, G.C.; Dong, X.H.; Chen, G.; Tan, B.P.; Yang, Q.H.; Chi, S.Y. and Liu, H.Y. (2015).** Physiological responses and HSP70 mRNA expression of GIFT strain of Nile tilapia (*Oreochromis niloticus*) under cold stress. *Aquac. Res.*, **46**: 658–668. <https://doi.org/10.1111/are.12212>.

- Sifa, L.; Chenhong, L.; Dey, M.; Gagalac, F. and Dunham, R. (2002).** Cold tolerance of three strains of Nile tilapia, *Oreochromis niloticus*, in China. *Aquaculture*, 213: 123–129.
- Sun, L.T.; Chen, G.R. and Chang, C.F. (1992):** The physiological-responses of tilapia exposed to low temperatures. *J. Therm. Biol.*, **17**: 149–153.
- Younis, E.M. (2015) :** Variation in metabolic enzymatic activity in white muscle and liver of blue tilapia, *Oreochromis aureus*, in response to long-term thermal acclimatization. *Chin. J. Oceanol. Limnol.*, **33**: 696-704.