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# Effect of fish overwintering in earthen ponds and RAS treatment on growth performance of the Nile tilapia, *Oreochromis niloticus*

**Hany Maher[1], Ahmad M. Azab[1*], Ismail A. Radwan[2] and Mohamed Sh. Abu Husein[1]**

1- Zoology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt
2- Egyptian Aquaculture Centre for training and applied research, Kafr El-Sheikh, Egypt.
*Corresponding author: amazab2000@yahoo.com

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## ABSTRACT

The present work aimed to enhance the tolerance of juvenile Nile tilapia, *Oreochromis niloticus* against cold stress and increase their survival rate by using the recirculating aquaculture system (RAS) during the winter season. The overwintering experiment was carried out in 3 earthen ponds, each of 1 feddan (as replicates of the control group); and 3 circular fiberglass tanks at an intensive fish farm, located in Nubaria City, Beheira Governorate, Egypt. The indoor 1000-liter circular fiberglass tanks (as replicates of recirculating aquaculture system, RAS treatment group). The experiment extended from December 2018 to April 2019. Results showed that the Nile tilapia fingerling reared in the recirculating aquaculture system (RAS) exhibited values of growth performance parameters ($L_f$, LG, DLG and G in L, $W_f$, WG, DWG, G in W and SGR) higher than that of fish fingerlings reared in control earthen ponds. The growth performance parameters were significantly varied between all tanks of the RAS treatment and that of control ponds. Also, the feed utilization parameters were best for fishes reared in RAS treatment tanks than that reared in earthen ponds. The survival rate recorded in RAS aquaculture was very high (90.0± 5.0 %) and it was very low (17.1± 1.474 %) in control earthen ponds. The economic analysis showed positive net income values (1080, 840 & 600 LE) for RAS Tanks 2, 1 & 3, respectively. But, negative net income values were recorded (-34200, -30650 & -30010LE)for earthen ponds 3,2 & 1, respectively.

## INTRODUCTION

In the world, the Nile tilapia is the most important cultured fish species; after carp fishes. This species has several traits that make it a great choice for aquaculture; including its rapid growth, tolerance to a wide range of environmental conditions, resistance to stress and disease, ability to reproduce in captivity, feeding at low dietary levels and acceptance of artificial feed immediately after sucking the yolk sac. Thus, it is recently cultured in most of the world's countries; where the global tilapia production has rebounded from less than 0.5 million metric tons in the early 1990s to 6.03 million metric tons in 2018, with an average annual growth rate of 13.5% **(Wang *et al*., 2016; El-Sayed, 2020; FAO, 2020)**.

Tilapia is a tropical fish that is well adapted to warm environmental condition. They cannot tolerate low temperatures (below 10 degrees Celsius) for more than a few days. So, the main obstacle to tilapia farming is their sensitivity to sharp drops in water temperatures during the winter months. This is a major economic concern as it causes weak growth. As a result, mass deaths of tilapia were recorded during the winter season in some subtropical regions, such as in Egypt. Therefore, enhancing the tolerance of Nile tilapia to cold temperature is important to prolong their growth period, reduce mortality during excessive winters and reduce economic losses **(Sun *et al*., 1992; Costa-Pierce, 2003; Charo-Karisa *et al*., 2005; El-Sayed, 2006; Ernst *et al*., 2007; El-Sayed & Kawanna, 2008; Hassan *et al*., 2013)**.

Young fish in the temperate latitudes face multiple sources of death during their first time of life. Starvation and physiological shock are the two main sources of mortality during the winter season. In winter season, juveniles often switch from maximizing growth to maximizing energy stores to prevent starvation during this cold season **(Schultz & Conover, 1999; Post & Parkinson, 2001; Hurst & Conover, 2003; Biro *et al*., 2005; Heintz & Vollenweider, 2005; Hurst, 2007)**.

New methods must be found to tolerate the young farmed fish in the cold season and to increase their survival rate during winter. Several strategies have been implemented to provide suitable temperature in tilapia ponds during winter by using heating and geothermal water facilities and plastic sheeting the ponds **(Dan & Little, 2000 a & b; Yokoyama *et al*., 2005; Abdel-Aal, 2008)**.

Therefore, the present work aimed to enhance the tolerance of juvenile Nile tilapia, *Oreochromis niloticus* against cold stress and increase their survival rate by using the recirculating aquaculture system (RAS) during the winter season.

## MATERIALS AND METHODS

### 1. Overwintering experimental design:

The experiment of overwitering was carried out in earthern ponds as a control group and in recirculating aquaculture system (RAS) tanks as a treatment group.

### 1.1. Earthern ponds:

The control experimental group was carried out in 3 earthern ponds at 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 with an approximately average depth of 1.25m and filled in through drainage 7.

### 1.2. Recirculating aquaculture system (RAS) tanks:

The recirculating aquaculture system (RAS) treatment was carried out at an intensive fish farm, located in at in the Aquaculture Research Unit, Cairo Feed Company, Nubaria City, Beheira Governorate, Egypt. The indoor 1000-liter circulars fiberglass tanks with a RAS system. The rearing system consisted of a mechanical sand filter (Hayward, USA), a biofilter tank (1000 L with plastic media) and ultraviolet lights (Philips, the Netherlands). Water was supplied at a rate of --- l/h and thermos regulated heaters (4 KW) in water supply sump to constant the temperature of 28 ± 1 °C. The photoperiod was 12 h light/ 12 h darkness through artificial light. The oxygen level was kept above 6 mg/l, salinity (3 g/l) and pH constant at 7.5 to 8.2 measured by Nilebot water quality monitoring system. Ammonia (total ammonia nitrogen $\leqslant$0.1 ppm) and nitrite ($\leqslant$0.2 ppm) were spectrophotometrically monitored weekly.

### 1.3. Experimental fish grouping and stocking:

The experimental fish was the monosex of Nile tilapia, *Oreochromis niloticus* fingerlings. The experiment extended from December 2018 to April 2019 (112 days). The experimental fish diet (floating pellets in RAS tanks and sinking pellets in earthern ponds) was of the commercial type (30% protein), bought from Cairo Feed Company, Cairo Poultry Corporation (CPC).

The control group was carried out in three replicate earthern ponds. They stocked by 315000, 310000 and 260000 monosex fries of Nile tilapia in pond 1 ($P_1$), pond 2 ($P_2$) and pond 3 ($P_3$), respectively. These fries were reared in ponds from September 2018 till December 2018. At the beginning of overwintering experiment, they reached to fingerlings with an average length of 4.30± 0.491cm and average weight of 3.74± 1.177 g.

The treatment experiment group was carried out in three tanks as RAS replicates ($T_1$, $T_2$ and $T_3$). Each tank was stocked by 3000 fingerlings monosex of Nile tilapia, with an average length of 6.04±0.263 cm and average weight of 7.59± 0.97 g.

## 2. Growth in length parameters:

Growth in length, length gain, growth in weight, total weight gain, average daily weight gain, specific growth rate, feed intake (FI) and feed conversion ratio in juveniles of *Oreochromis niloticus* were determined according to **Recker, (1975)** and **Castell &Tiews, (1980)** as following:

### 2.1. Final body length (cm):

The fish length of each fish, from control and treatment groups, was recorded at the end of the experiment.

### 2.2. Total length gain (cm/fish):

The length gain is calculated from the following equation:

$$\mathbf{L\,G = L_f - L_i}$$

Where: $\mathbf{L_f}$= final average length of fish in cm.

$\mathbf{L_i}$ = initial average length of fish in cm.

### 2.3. Daily length gain (µ/fish/day):

The average daily length gain is calculated from the following equation:

**DLG = total length gain (µ)/ duration period (days)**

### 2.4. Growth in length (%):

The growth in length is calculated from the following equation:

$$\mathbf{G\ in\ L = \{LG / L_i\} \times 100}$$

Where: **LG**= Total length gain (cm).

$\mathbf{L_i}$ = Initial average length of fish (cm).

## 3. Growth in weight parameters:

### 3.1. Final body weight (g):

The fish weight of each fish, from control and treatment groups, was recorded at the end of the experiment.

**3.2. Total weight gain (g/fish):**

The total weight gain is calculated from the following equation:

$$\textbf{Total weight Gain} = W_f - W_i$$

Where: $W_f$ = final means weight of fish in grams.

$W_i$ = initial means weight of fish in grams.

**3.3. Average daily weight gain (mg /fish/day):**

The average daily weight gain is calculated from the following equation:

**DWG = total weight gain (mg)/ duration period**

**3.4. Growth in weight (%):**

The growth in weight is calculated from the following equation:

$$\textbf{G in W} = \{WG / W_I\} \times 100$$

Where: **WG**= Total weight gain (g).

$L_I$ = Initial average weight of fish (g).

**3.5. Specific growth rate (% / day):**

The specific growth rate is calculated from the following equation:

$$SGR = (\ln W_f - \ln W_i) * 100 / \text{duration period}$$

Where:

**Ln**= Natural log.

$W_f$ = final means weight of fish in grams.

$W_i$ = initial means weight of fish in grams.

**4. Feed utilization parameters:**

**4.1. Total Feed intake:**

The total feed intake is calculated from the following equation:

$$FI = \sum\{\text{monthly average fish weight} * (\text{daily feeding rate} * 25)\}$$

**4.2. Food conversion ratio:**

The feed conversion ratio is calculated from the following equation:

**FCR = feed intake (g)/ total weight gain (g)**.

**4.3. Feed efficiency (FE):**

The feed efficiency is calculated by the following equation:

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

**4.4. Total protein intake (PI):**

The total feed intake is calculated from the following equation:

**PI = feed intake (g) * Protein% in the diet/100**

**4.5. Protein efficiency ratio (PER):**

The protein efficiency ratio is calculated by the following equation:

**PER = total gain (g)/ protein intake (g)**

**5. Survival rate (SR, %):**

The survival rate is calculated by the following equation:

$$\mathbf{SR\ (\%) = N_f/N_i * 100}$$

Where: $\mathbf{N_f}$= Number of fish survived at the end of the experiment

$\mathbf{N_i}$= Number of fish stocked at the start of the experiment

**6. Economic evaluation:**

Total coasts were calculated by the following equation:

**Total costs= feed costs (LE) + fish fry cost (LE)+ operation cost (LE)**

Operation costs include workers, electricity, service…etc. All experimental diet costs, fish fry cost and operation cost were calculated according to the prices in Egyptian market during the study period.

The economic evaluation was calculated by the following equation:

**Net income (LE) = Total fish price (LE) - Total costs (LE)**

**7. Statistical analysis:**

Statistical analysis and graphics of data was 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 by Holm-Sidak Method **(Holm, 1979)** at 95% confidence.

## RESULTS

The effect of fish overwintering in earthen aquaculture (Ponds 1 - 3) and RAS aquaculture (Tanks 1 - 3) 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), specific growth rate (SGR %/day); feed intake (FI g/fish), feed conversion ratio (FCR), protein efficiency ratio (PER/g) and feed efficiency (FE) of *Oreochromis niloticus,* are given in **Tables (1 - 5)** and are graphically represented in **Figs. (1 - 8)**.

**1. Growth in length parameters:**

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

Results showed that, *Oreochromis niloticus* fingerlings reared in earthen ponds and RAS aquacultures exhibited great variations in body length. The highest average body length (**11.61± 0.57** cm) was recorded in RAS aquaculture, representing average growth in length (**98.8± 10.856%**). While, the lowest average body length (**7.28± 0.938** cm) was recorded in earthen ponds, representing the average growth in length of **77.3± 13.154**% **(Tables, 1 & 2** and **Fig. 1)**.

**1.2. Length gain (LG, cm/fish):**

Results showed that *O. niloticus* fingerlings reared in earthen ponds and RAS aquacultures exhibited greatly variation in length gain. The greatest length gain (5.57± 0.614 cm) was recorded in RAS aquaculture and the length gain in fishes of earthen ponds was 2.98± 0.653 cm **(Table, 2** and **Fig. 1)**.

### 1.3. Average daily length gain (DLG, µ/fish/day);

Results showed that, *O. niloticus* fingerlings reared to overwinter in both of earthen ponds and RAS aquacultures exhibited greatly variation in daily length gain. The daily length gain of fishes reared in earthen ponds was 266.3 ± 58.263 µ/fish/day and the length gain of fishes in RAS aquaculture was 498± 54.81µ/fish/day**(Table, 2** and **Fig. 1)**.

The results also showed that the growth performance parameters in length ($L_f$, LG, DLG and G in L) were significantly varied between all tanks of the Recycle Aquaculture System (RAS) treatment and that of control ponds.

For growth in length, length gain LG and daily length gain (DLG), $Tank_1$ was significantly varied ($P< 0.05$) in comparison to other tanks and ponds. Also, $Tank_2$ and $Tank_3$ were significantly varied in comparison with all ponds.

**Table (1): Total fish length in cm (average ± SD) of *O. niloticus*, for control and Recycle Aquaculture System (RAS) treatment during winter months**

| Treatments | | Months | | | | |
|---|---|---|---|---|---|---|
| | | December 2018 (Zero-day) | January 2019 | February 2019 | March 2019 | April 2019 (Final day) |
| Control | Pond 1 | 3.73±0.885 | 4.47±0.746 | 5.19±0.608 | 5.72±0.507 | 6.44±0.767 |
| | Pond 2 | 4.60±1.040 | 5.04±0.718 | 5.86±0.636 | 6.72±0.675 | 7.11±0.644 |
| | Pond 3 | 4.57±0.844 | 5.14±0.735 | 5.70±0.667 | 7.83±0.629 | 8.29±0.694 |
| Totals | | **4.30± 0.491** | **4.88±0.733** | **5.52±0.637** | **6.76±0.604** | **7.28±0.938** |
| RAS Treatment | Tank 1 | 5.87±0.98 | 6.26±1.063 | 9.30±1.625 | 10.72±2.33 | 12.14±1.98 |
| | Tank 2 | 6.34±1.141 | 6.93±1.356 | 8.80±1.743 | 10.69±1.63 | 11.7±2.146 |
| | Tank 3 | 5.90±1.032 | 6.08±0.923 | 9.58±1.542 | 10.97±1.14 | 11.0±2.402 |
| Totals | | **6.04±0.263** | **6.42±0.448** | **9.23±0.393** | **10.79±0.16** | **11.61±0.57** |

**Table (2): Growth performance parameters in length (average± SD) of *O. niloticus*, for control and Recycle Aquaculture System (RAS) treatment during winter months**

| Treatments | | Initial length (cm) | Final length (cm) | Length gain (cm/fish) | Daily length gain (µ/fish/day) | Growth in length (%) |
|---|---|---|---|---|---|---|
| Control | Pond 1 | $3.73^{d}$± 0.885 | $6.44^{b}$± 0.767 | $2.71^{cd}$± 1.123 | $241.9^{cd}$± 100.28 | $81.7^{cd}$± 45.93 |
| | Pond 2 | $4.60^{d}$± 1.040 | $7.11^{b}$± 0.644 | $2.51^{d}$± 1.251 | $224.1^{d}$± 111.72 | $62.5^{d}$± 40.27 |
| | Pond 3 | $4.57^{cd}$± 0.844 | $8.29^{b}$± 0.682 | $3.73^{c}$± 0.984 | $332.7^{c}$± 87.89 | $87.7^{bc}$± 36.81 |
| Totals | | **4.30± 0.491** | **7.28± 0.938** | **2.98± 0.653** | **266.3± 58.263** | **77.3± 13.154** |
| RAS Treatment | Tank 1 | $5.87^{ab}$± 0.98 | $12.14^{a}$± 1.98 | $6.27^{a}$± 2.03 | $559^{a}$**± 180.82** | $111.3^{a}$± 45.37 |
| | Tank 2 | $6.34^{a}$± 1.141 | $11.7^{a}$± 2.146 | $5.34^{b}$± 2.554 | $477^{b}$± 228.0 | $91.4^{bc}$± 52.176 |
| | Tank 3 | $5.90b^{c}$±1.032 | $11.0^{a}$± 2.402 | $5.11^{b}$± 2.694 | $456^{b}$± 240.6 | $93.7^{b}$± 60.405 |
| Totals | | **6.04*± 0.263** | **11.61*± 0.57** | **5.57*± 0.614** | **498*± 54.81** | **98.8*± 10.856** |

*****means significantly varied (p<0.05); Different letters in the same column is significantly varied (P<0.05)

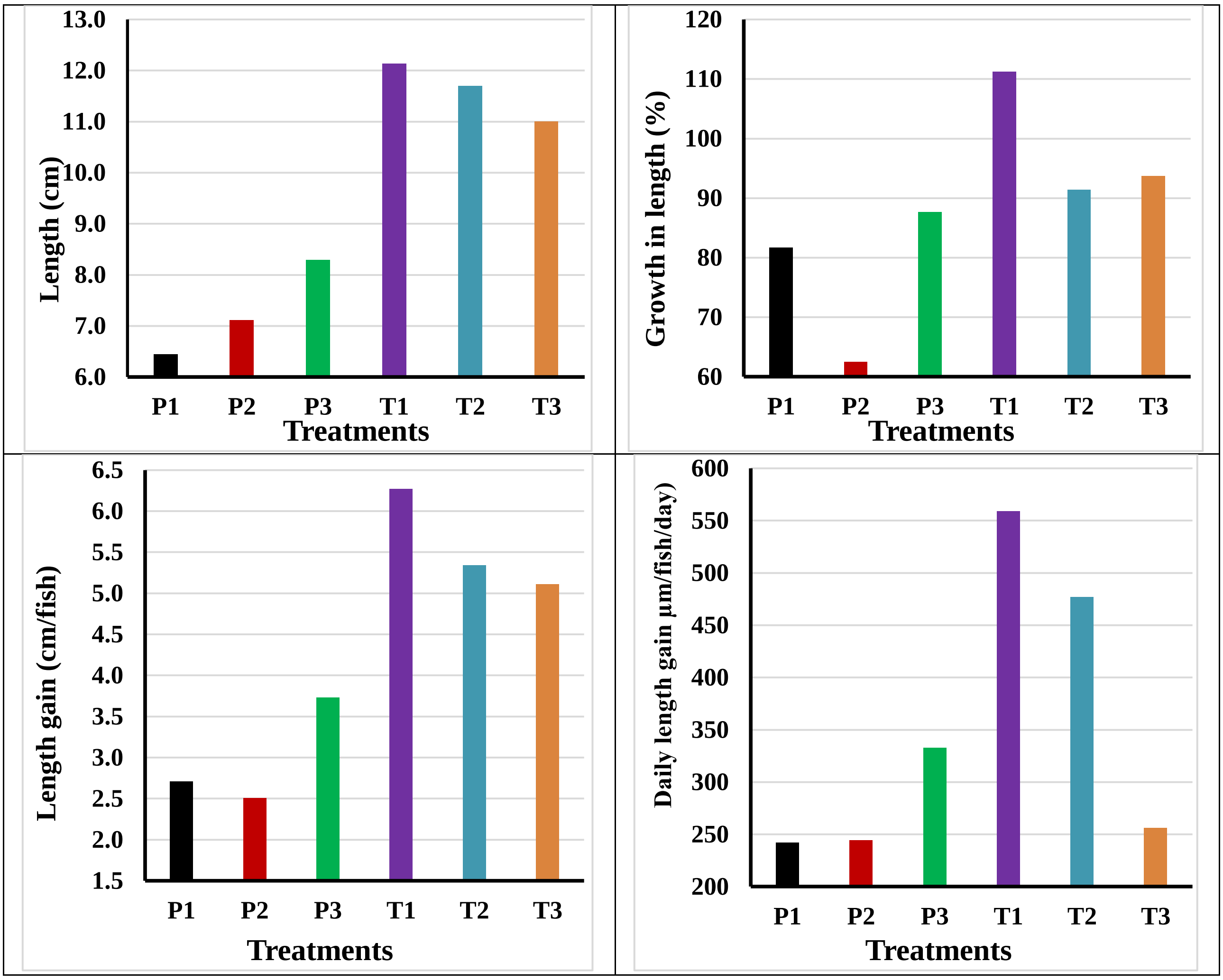


**Fig. (1).** Effect of *O. niloticus* fingerlings overwintering in control ponds (P1, P2 and P3) and Recycle Aquaculture System (RAS) tanks (T1, T2 and T3) on length growth parameters.

## 2. Growth in weight parameters:

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

Results in **Tables (3&4) and Fig. (2)** showed that, *Oreochromis niloticus* fingerlings reared with different earthen ponds and RAS aquaculture tanks exhibited great variations in body weight. The highest average body weight (39.02± 2.84 g) was recorded in RAS aquaculture, while the lowest average body weight (11.8± 5.451 g) was recorded in earthen ponds. The highest growth in weight (649± 50.34%) was recorded for RAS aquaculture, while the lowest growth in weight (237.2± 81.91%) recorded for earthen ponds.

### 2.2. Total weight gain (WG, g/fish):

Results showed that *O. niloticus* fingerlings reared with different earthen ponds and RAS aquaculture exhibited greatly variation in weight gain. The greatest weight gain (31.43± 2.66 g) was recorded in RAS aquaculture and the lowest weight gain (8.07± 4.583 g) was recorded in earthen ponds **(Table 4** and **Fig. 2)**.

### 2.3. Average daily weight gain (DWG, mg/fish/day):

Results showed that, *O. niloticus* fingerlings reared with different earthen ponds and RAS aquaculture exhibited greatly variation in daily weight gain. The greatest weight gain

(281± 23.78 mg/fish/day) was recorded in RAS aquaculture and the lowest daily weight gain (72.0± 40.92 mg/fish/day) was recorded in earthen ponds **(Table 4** and **Fig. 2)**.

### 2.4. Specific growth rate (% / day):

The results showed that, *O. niloticus* fingerlings reared with different earthen ponds and RAS aquaculture exhibited great variations in specific growth rate. The highest average specific growth rate (1.85± 0.074%) was recorded in RAS aquaculture. While, the lowest average specific growth rate (1.27± 0.246 %) was recorded in earthen ponds **(Table 4)**.

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

For final weight ($W_f$), growth in weight (G in W) and SGR, $Tank_1$ was significantly varied ($P< 0.05$) in comparison to other tanks and ponds. Also, $Tank_2$ and $Tank_3$ were significantly varied in comparison with all ponds. The other parameters of growth performance in weight were statistically varied between all tanks and that of ponds

**Table (3): Growth in weight in grams (average ± SD) of *O. niloticus*, for control and Recycle Aquaculture System (RAS) treatment during winter months**

| Treatments | | Months | | | | |
|---|---|---|---|---|---|---|
| | | December (Zero-day) | January | February | March | April (Final day) |
| Control | Pond 1 | 2.39± 0.685 | 3.40± 0.714 | 4.39± 0.727 | 5.82± 1.060 | 7.21± 1.603 |
| | Pond 2 | 4.31± 1.259 | 4.78± 1.199 | 5.26± 1.140 | 9.41± 1.863 | 10.37± 1.46 |
| | Pond 3 | 4.52± 1.185 | 5.37± 1.220 | 6.21± 1.255 | 15.06± 3.25 | 17.83± 3.91 |
| **Totals** | | **3.74± 1.177** | **4.52± 1.011** | **5.29± 0.910** | **10.1± 4.654** | **11.8± 5.451** |
| RAS Treatment | Tank 1 | 7.46± 4.20 | 9.03± 4.681 | 15.61± 9.31 | 26.3± 15.54 | 41.9± 18.53 |
| | Tank 2 | 8.62± 5.368 | 12.39± 6.30 | 14.64± 7.99 | 25.7± 10.49 | 38.1± 18.03 |
| | Tank 3 | 6.69± 3.637 | 8.38± 4.219 | 17.75± 8.50 | 27.62± 9.57 | 36.3± 21.00 |
| **Totals** | | **7.59± 0.97** | **9.93± 2.157** | **16.0± 1.588** | **26.5± 0.977** | **39.02± 2.84** |

**Table (4): Growth performance parameters in weight (average ± SD) of *O. niloticus*, for control and Recycle Aquaculture System (RAS) treatment during winter months**

| Treatments | | Initial weight (g) | Final weight (g) | Weight gain (cm/fish) | Daily weight gain (mg/fish/day) | Growth in weight (%) | Specific growth rate (%/day) |
|---|---|---|---|---|---|---|---|
| Control | Pond 1 | $2.39^{d}$± 0.685 | $7.21^{e}$± 1.603 | $4.83^{b}$± 1.785 | $43.1^{b}$± 15.94 | $224.9^{d}$± 108.2 | $1.21^{d}$± 0.34 |
| | Pond 2 | $4.31^{c}$± 1.259 | $10.37^{d}$± 1.46 | $6.06^{b}$± 2.071 | $54.1^{b}$± 18.49 | $162.1^{d}$ ± 85.36 | $1.06^{d}$± 0.31 |
| | Pond 3 | $4.52^{bc}$± 1.185 | $17.83^{c}$± 3.91 | $13.31^{b}$± 4.185 | $118.8^{b}$± 37.36 | $325.0^{c}$± 148.0 | $1.54^{c}$± 0.34 |
| **Totals** | | **3.74± 1.177** | **11.8± 5.454** | **8.07± 4.583** | **72.0± 40.92** | **237.2 ± 81.91** | **1.27± 0.246** |
| RAS Treatment | Tank 1 | $7.46^{a}$± 4.20 | $41.9^{a}$± 18.53 | $34.5^{a}$± 18.13 | $308^{a}$± 161.8 | $617^{a}$± 492.5 | $1.94^{a}$± 0.68 |
| | Tank 2 | $8.62^{a}$± 5.368 | $38.1^{ab}$± 18.03 | $30.2^{a}$± 19.55 | $270^{a}$± 174.5 | $707^{b}$± 806.6 | $1.81^{b}$± 0.97 |
| | Tank 3 | $6.69^{ab}$± 3.637 | $36.3^{b}$± 21.00 | $29.6^{a}$± 21.11 | $264^{a}$± 188.5 | $623^{b}$± 655.9 | $1.81^{b}$± 0.83 |
| **Totals** | | **7.59*± 0.97** | **39.0*± 2.84** | **31.43*± 2.66** | **281*± 23.78** | **649*± 50.34** | **1.85*± 0.074** |

*****means significantly varied (p<0.05); Different letters in the same column is significantly varied (P<0.05)

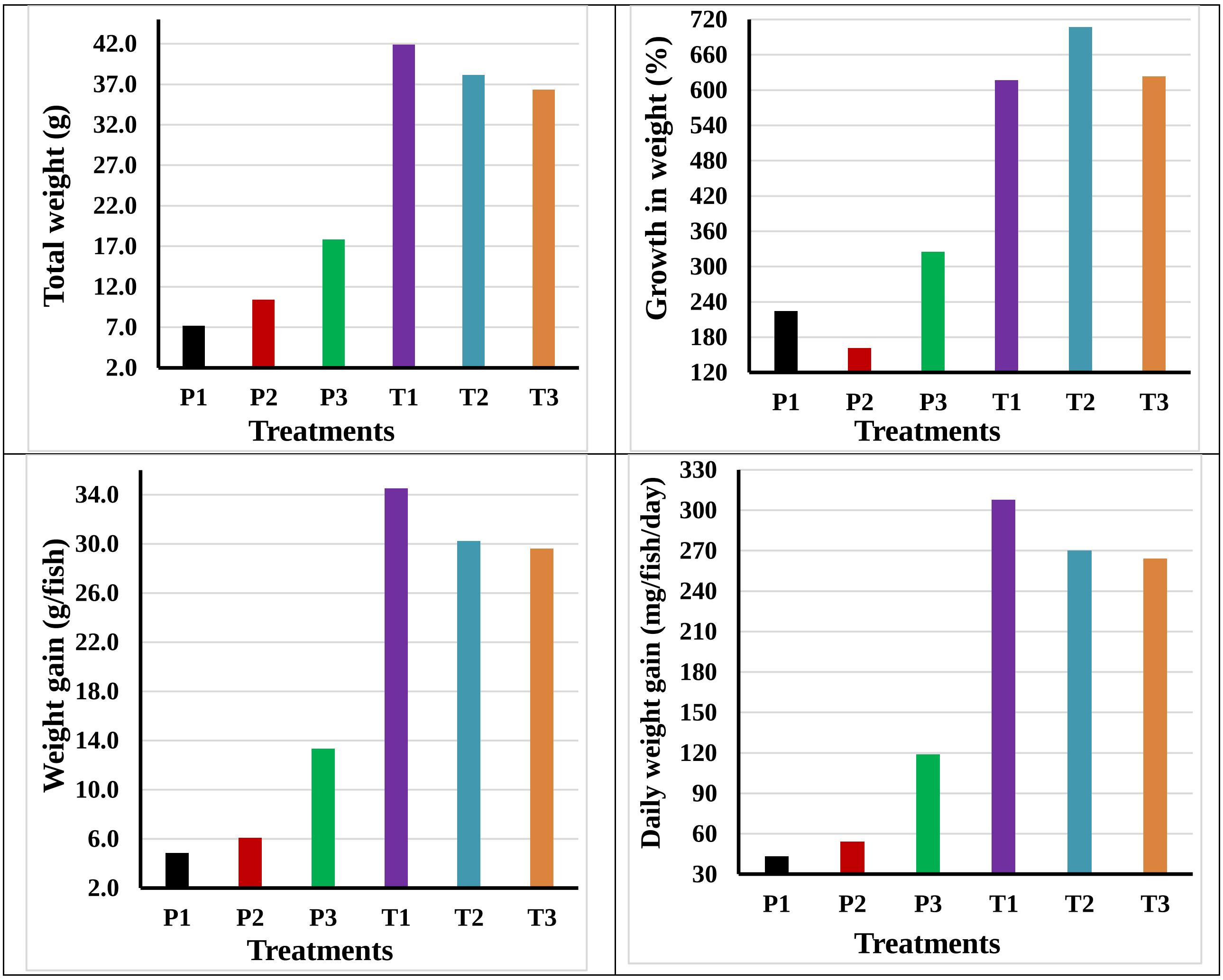


**Fig. (2).** Effect of *O. niloticus* fingerlings overwintering in control ponds (P1, P2 and P3) and Recycle Aquaculture System (RAS) tanks (T1, T2 and T3) on weight growth parameters.

## 3. feed utilization parameters:

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

Results in **Table (5)** showed that, *O. niloticus* fingerlings reared with different earthen ponds and RAS aquaculture exhibited great variations in feed intake. The highest average feed intake (43.40±7.910 g) was recorded in earthen ponds. The lowest average feed intake (37.86±2.106 g) in RAS aquaculture.

### 3.2. Feed conversion ratio:

Results showed that, *O. niloticus* fingerlings reared with different earthen ponds and RAS aquaculture exhibited great variations in feed conversion ratio. The best feed conversion ratio (1.21± 0.130) was recorded in RAS aquaculture. The worst feed conversion ratio (6.12± 2.012) was record in earthen ponds **(Table 5)**.

### 3.3. Feed efficiency (FER):

Results showed that, *Oreochromis niloticus* fingerlings reared earthen ponds and RAS aquaculture exhibited great variations in feed efficiency ratio. The highest feed efficiency ratio (0.83±0.087) was recorded in RAS aquaculture and the lowest (0.18±0.067) occurred in earthen ponds **(Table 5)**.

### 3.4. Protein intake (PI / g):

Results showed that, *O. niloticus* fingerlings reared earthen ponds and RAS aquaculture exhibited great variations in protein efficiency ratio. The highest protein intake (13.02± 2.373g) was recorded in earthen ponds and the lowest (11.36± 0.632 g) was recorded for RAS aquaculture **(Table 5)**.

### 3.5. Protein efficiency ratio (PER):

Results showed that, *Oreochromis niloticus* fingerlings reared earthen ponds and RAS aquaculture exhibited great variations in protein efficiency ratio. The highest protein efficiency ratio (2.77± 0.290 g) was recorded in RAS aquaculture and the lowest (0.87± 0.252 g) 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**

| Treatments | | Feed intake (g/fish) | Feed conversion ratio | Feed efficiency ratio | Protein intake (g/fish) | Protein efficiency ratio |
|---|---|---|---|---|---|---|
| Control | Pond 1 | 38.158 | 7.908 | 0.126 | 11.447 | 0.621 |
| | Pond 2 | 39.545 | 6.522 | 0.153 | 11.864 | 0.866 |
| | Pond 3 | 52.500 | 3.944 | 0.254 | 15.750 | 1.126 |
| **Totals** | | **43.40± 7.910** | **6.12± 2.012** | **0.18± 0.067** | **13.02± 2.373** | **0.87± 0.252** |
| RAS Treatment | Tank 1 | 37.78 | 1.095 | 0.913 | 11.333 | 3.043 |
| | Tank 2 | 35.79 | 1.185 | 0.844 | 10.737 | 2.814 |
| | Tank 3 | 40.00 | 1.351 | 0.740 | 12.000 | 2.467 |
| **Totals** | | **37.86± 2.106** | **1.21± 0.130** | **0.83± 0.087** | **11.36± 0.632** | **2.77± 0.290** |

## 4. Survival rate (SR):

Results in **Table (6)** showed that, *Oreochromis niloticus* fingerlings reared earthen ponds and RAS aquaculture exhibited great variations in survival rate. The highest survival rate (90.0± 5.0 %) was recorded in RAS aquaculture and the lowest (17.1± 1.474 %) was recorded for earthen ponds.

**Table (6): Survival ratio of *O. niloticus*, for control and Recycle Aquaculture System (RAS) treatment during winter months**

| Treatments | | Initial number | Survival | |
|---|---|---|---|---|
| | | | Fish Number | % |
| Control | Pond 1 | 315000 | 57000 | 18.1 |
| | Pond 2 | 310000 | 55000 | 17.7 |
| | Pond 3 | 260000 | 40000 | 15.4 |
| **Totals (Average ± SD)** | | | | **17.1± 1.474** |
| RAS Treatment | Tank 1 | 3000 | 2700 | 90.00 |
| | Tank 2 | 3000 | 2850 | 95.00 |
| | Tank 3 | 3000 | 2550 | 85.00 |
| **Totals (Average ± SD)** | | | | **90.0± 5.00** |

## 5. Economical evaluation:

Results in **Table (7)** indicated that, the total feed intake in the experiment of Nile Tilapia, *O. niloticus* (2100 kg) was recorded for earthern ponds 1, 2 and 3. The total feed intake used in RAS aquaculture was 102 kg for Tanks 1, 2 and 3**.**

The results of **Table (7)** showed that the total costs including feed cost, fish fry cost and operation costs were 62500, 62000 and 57000 L.E for earthern ponds 1, 2 and 3, respectively. In case of RAS aquaculture experiment, the total costs were 3480 L.E for each tank.

The total fish price is calculated by summation the prices of 1000 fish according to its size **(Table 7)**. The maximum total fish price (32490 L.E) was recorded for earthern pond **1**. It decreases gradually at Pond 2 (31350 L.E), followed by Pond 3 (22800 L.E) and the lowest value at RAS aquaculture Tank 3 (4080 L.E), Tank 1 was record (4320 L.E) and Tank 2 was record (4560 L.E) **(Table 7).**

Results in **Table (7)** shows that the positive net income recorded the maximum value (1080 LE) in RAS aquaculture Tank 2, followed by Tank 1 (840 LE); it followed by Tank 3 (600 LE). The negative net income recorded the maximum value in earthen ponds at Pond 3 (-34200 LE) followed by (-30650 LE) at Pond 2, and Pond 1 recorded -30010 L.E.

**Table (7): The economic evaluation of *O. niloticus* production, for Earthen ponds and RAS treatments during winter months**

| Subject | | Unit price (LE) | Control | | | RAS treatment | | |
|---|---|---|---|---|---|---|---|---|
| | | | Pond 1 | Pond 2 | Pond 3 | Tank 1 | Tank 2 | Tank 3 |
| Costs | Feed intake (Kg) | | 2100 | 2100 | 2100 | 102 | 102 | 102 |
| | Feed cost (LE) | 10 | **21000** | **21000** | **21000** | **1020** | **1020** | **1020** |
| | Fish fry used (1000 fries) | Fries | 315 | 310 | 260 | | | |
| | | Fingerlings | | | | 3 | 3 | 3 |
| | Fish fry cost (LE) | 100 | 31500 | 31000 | 26000 | | | |
| | | 420 | | | | 1260 | 1260 | 1260 |
| | Operation cost (LE) * | | 10000 | 10000 | 10000 | 1200 | 1200 | 1200 |
| | Total costs (LE) | | **62500** | **62000** | **57000** | **3480** | **3480** | **3480** |
| Income | Final fish no. (1000 fish) | | 57 | 55 | 40 | | | |
| | | | | | | 2.70 | 2.85 | 2.55 |
| | Total income (LE/1000 fish) | 570 | **32490** | **31350** | **22800** | | | |
| | | 1600 | | | | **4320** | **4560** | **4080** |
| Net income (LE) | Whole | Total | **-30010** | **-30650** | **-34200** | **840** | **1080** | **600** |
| | | Average | **-31620** | | | **840** | | |
| | Per 1000 fingerlings | Total | **-95.3** | **-98.9** | **-131.5** | **280** | **360** | **200** |
| | | Average | **-108.6** | | | **280** | | |

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

## DISCUSSION

Cold stress is a primary contributing factor to fish diseases and mortality, and thus, this has resulted in decreased production of aquaculture systems in the tropical region. Moreover, cold stress has negative effects on fish such as reducing metabolic rates, swimming performance, and harming immune function. In relation to the Nile tilapia, *Oreochromis niloticus* as a warm tropical water fish is able to tolerate a wide range of temperatures (8-42 °C). It does not grow well below 16°C and does not survive below 10°C for more than a few days. Activity and feeding decrease when the temperature drops below 20°C and stop completely at around 16°C. The winter temperature dropped to 16 °C or less, which negatively affected the survival and growth of tilapia cultured in aquaculture ponds in Egypt **(Hocutt, 1973; Galloway & Kieffer, 2003; Bauer & Schlott, 2004; Hurst, 2007; Siddik *et al*., 2014; Soltan *et al*., 2015; Hassaan *et al*., 2019; Wu *et al*., 2019)**.

The present work showed that in the erthern ponds the survival rate was very low (17.1± 1.474 %) 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 previous studies, where the temperature fluctuated from 2 to 14 ºC at night during January and February 2019 and this resulted in a high mortality rate. On the other hand, the survival rate in RAS tanks was very high (90.0± 5.00). where the temperature in the farm was adjusted to 28±1 °C.

**Table (8): Variation in air temperatures ºC during winter months 2018-2019 (**After: **Weather and Climate, 2023)**

| Months | Air temperatures (ºC) | | | |
|---|---|---|---|---|
| | Minimum | | Maximum | |
| | Range | Average ±SD | Range | Average ±SD |
| **December 2018** | 09 - 15 | 12.13±1.28 | 15 - 23 | 19.65±1.9 |
| **January 2019** | 02 - 10 | 07.06±1.78 | 14 - 22 | 17.74±1.99 |
| **February 2019** | 02 - 14 | 8.79±2.39 | 14 - 27 | 18.00 ±2.98 |
| **March 2019** | 07 - 16 | 11.35±2.50 | 15 - 25 | 19.00±2.05 |
| **April 2019** | 09 - 21 | 13.87±2.64 | 17 - 39 | 23.63±4.76 |

The present results showed that the fingerlings of Nile tilapia reared in RAS aquaculture tanks exhibited highest averages of growth performance parameters (growth in length, length gain, daily length gain, growth in weight, total weight gain, daily weight gain, specific growth rate) and the 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 growth performance 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 over-wintering fish reared in concrete pond.

Successful over-wintering of tilapia in ponds has been conducted using heated facilities, geothermal water and greenhouses insulated with plastic sheet covers **(Dan & Little, 2000 a & b)**. A practical way to provide a proper temperature in the ponds is to cover the ponds with plastic sheets to preserve the temperature in the water and to absorb the solar radiation for heating **(Rothbard & Peretz, 2002)**.

The economic evaluation data showed recorded a positive net income with an average of 280 LE /1000 fingerlings in RAS aquaculture tanks and a negative net income with an average of -108.6 LE /1000 fingerlings in earthen ponds. So, the present work recommened to use the recirculating aquaculture system in overwintering the fries and fingerlings during the winter months to decrease the fish mortality.

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