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Effect of different concentrations of Ethylenediamine tetraacetic acid (EDTA) on growth performance of Nile tilapia, *Oreochromis niloticus* fry

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ABSTRACT

The present study deals with the effect of different concentrations of EDTA (0 g EDTA/l as a control, 0.1 g EDTA /l & 0.3 g EDTA /l) on the growth in length, growth in weight, survival rate, length-weight relationship, condition factors and growth performance of the cichlid fish, *Oreochromis niloticus* fry reared in glass aquaria. The improvement of fish fry development obtained by EDTA is dose-dependent. Fry obtained from high concentration of EDTA (0.3 g EDTA /l) showed higher increase in length, weight and survival rate than those of low concentration of EDTA (0.1 g EDTA /l) compared to control. The growth of fish fry exposed to high concentration of EDTA (0.3 g EDTA /l), is nearly isometric. The b value is nearly ideal (2.92) and the correlation coefficient (r) is statistically highly significant (0.99). While, the growth of fish fry is negatively allometric, in fish control (2.77) and fish exposed to low concentration of EDTA (2.43). The b value is less than the ideal. The values of condition factors are equal and the values of relative condition factors are slightly differences among fish fry exposed to different concentrations of EDTA. The total weight gain, average daily gain and specific growth rate of *O. niloticus*, fry improved with the high concentration of EDTA compared with the fish exposed to low concentration of EDTA and control. The best feed conversion ratio (0.66) was recorded in the fish fry exposed to 0.3 g EDTA/l compared with the fish control (0.8) and fish fry exposed to 0.1 g EDTA/l (1.02). In conclusion, the growth performance of Nile tilapia, *Oreochromis niloticus* fry are good pattern in the fry treated by 0.3 g EDTA /l. These observations suggest that, the exogenous treatment (0.3 g EDTA /l) may have practical utility in the fish culture.

Key words: EDTA; growth performance; *Oreochromis niloticus*

INTRODUCTION

From multitude of chemicals and drugs, disodium ethylene diamine tetraacetic acid (EDTA) is routinely used in aquaculture as a broad-spectrum "cure-all" in preventing metal toxicity to aquatic organisms. On the other hand, it is routinely used to keep certain essential metals in solution when needed for phytoplankton culture[(1)] or removing hardness and softening the hatching pond water[(2)]**.** EDTA is used in the intensive culture of penaeid shrimp larvae to increase both hatching rates[(3)] and survival of larvae[(3 , 4)]**.**

Fish importance as food source is increases with the increase in demands, especially in animal protein**.** In the recent years, a great attention has been paid to establishment of fish farms. These farms could contribute partially in producing the demanded on animal protein sources consumed by human[(5,6)]**.**

Tilapia is an ideal candidate for worm water aquaculture. They spawn easily in captivity, use a wide variety of natural foods as well as formulated feeds, tolerate poor water quality and grow rapidly at warm temperatures. These attributes, along with relatively low input costs have made tilapia widely cultured freshwater fish in tropical and subtropical countries[(7-14)]**.**

For the successful propagation of any new species, larval rearing is considered a major bottleneck[15,16]. During this period, transition from endogenous to exogenous feeding and weaning to artificial diets is characterized by high mortality rates, which are often linked to an inadequate food composition[17]. In Egyptian hatcheries, the transition from endogenous to exogenous nutrition often results in high mortalities of Nile tilapia larvae, which might be attributed to nutritional and infection problems[18].

The cause of mortalities may be physiological in nature given that starvation, due to the physical inability to feed after exhaustion of endogenous reserves. The period of mixed nutrition in rearing larvae of Nile tilapia, *O. niloticus* is considered as one of the most critical events during larval early life stages, since a several-day delay in feed availability influences posterior larval growth and survival. From the previous studies, two authors only investigate the improvement of growth performance in the Nile tilapia, *Oreochromis niloticus* by exogenous treatments, L-thyroxine (T4) hormone[19] and temperature[20]. However, there is no available information on the improvement of growth performance in the Nile tilapia by chelating agent, Ethylenediaminetetraacetic acid (EDTA).

Therefore, the aim of the present study to evaluate the effect of different concentrations of Ethylenediaminetetraacetic acid (EDTA) on growth performance of Nile tilapia, *Oreochromis niloticus* fry.

MATERIAL AND METHODS

A total of 1000 specimens Nile tilapia, *Oreochromis niloticus* fry, with a good condition obtained from fish hatchery of El-Hadad special farm at Kafr El-Sheikh governorate; during March, 2014. Fish transported to Cairo in large plastic bags filled with oxygen, each containing approximately 20 liters of fresh water. Duration of the journey was approximately three hours. Fish were acclimatized for 4 days in well aerated large glass tank. Fish were fed daily on a commercial fish diet.

In the laboratory fish were grouped randomly in 3 groups of 90 fish each; each fish group has 3 replicates which separated in experimental aquaria of 80 liters capacity (90X55X30 cm). Fish were exposed to 0 g EDTA /l as a control group, 0.1 g EDTA /l as treatment group 1, and 0.3 g EDTA /l as treatment group 2. The tanks were provided with aeration and thermostatic heater change of water weekly was carried out and the aquaria were cleaned and add one drop of 1% methylene blue for each tank. Fish in each aquarium were fed twice daily, seven days a week by commercial diet containing 65% protein, at a rate of 20 % of fresh body weight. The experiment was conducted for 5 weeks.

Biological Data:

1- Growth and survival rate:

Length and weight of 30 randomly fish from each treatment were weekly recorded and their initial, final weights and survival rate were recorded.

2- Length-weight relationship:

To study length-weight relationship, total length of each fish was measured to the nearest millimeter by steel ruler; and body weight was also determined to the nearest 0.0001 gram. Length-weight relationship of fry was determined by using the following equation[21]:

$W = a L^b$

Where:

W = Weight of the fish in milligram.

L = Total length in millimeter.
a and b = constants , whose values are estimated by the least square method.

3- Condition factors:

The length- weight relationship can be expressed in terms of condition factor (k), which measures the well-being of fish. Such factor was calculated from equation proposed by Hile[22]:

$K = 100\ W/\ L^3$

Where:
W = fish weight in milligram or gram.
L = fish length in millimeter or centimeter.

The relative condition factor (Kn) was determined by the following formula:

$Kn = W/w$

Where:
W = fish weight in milligram or gram.
w = calculated weight estimated from the length weight relationship equation.

4-Measurement of growth performance:

Total weight gain, average daily gain, specific growth rate, feed intake (FI) and feed conversion ratio in *O. niloticus* fry were determined[23, 24] as the following:

A- Total weight gain (mg/fish) = (W_T-W_I).
Where:
W_T = final means weight of fish in milligrams.
W_I = initial means weight of fish in milligrams.

B- Average daily gain (ADG) (mg/fish/day) = total gain/ duration period.

C- Specific growth rate (SGR) % / day) = $100\times(\text{Ln } W_T - \text{Ln } W_I)$/ duration period .
Where:
Ln= Natural log and n is the duration period in days.
D- Feed intake (FI) = fish weight x feeding level /100
E- Feed conversion ratio (FCR) = feed intake (mg)/ total weight gain (mg).

RESULTS

I- The effect of EDTA on growth and survival rate of *O. niloticus* fry:

The results in Table (1) showed that, *O. niloticus* fry exposed to EDTA (0.3 g/l) improved fry growth. Moreover, treatment with the high concentration of EDTA (0.3 g/l) over the rearing period of 35 days gave higher increase in length of fry (35.2±3.2mm) than control (0.0g/l EDTA) over the rearing period of 35 days (32.3±2.6mm) compared with the low concentration of EDTA (0.1 g/l) over the rearing period of 35 days (29.8±3.2).

Results in table (1) showed that, *O. niloticus* fry exposed to 0.3 g EDTA /l over the rearing period of 35 days exhibited greater average body weight (521.7±146.2 mg) than those of control (417.9±83.9 mg). Moreover, treatment with the low concentration of EDTA (0.1 g/l) over the rearing period of 35 days gave lower increase in weight of fry (328.5 ±106 mg) compared with the control.

Results in Table (2) showed that, *O. niloticus* fry exposed to EDTA (0.1 or 0.3 g/l) improved survival rate of fry than those of control. Moreover, treatment with the high concentration of EDTA (0.3 g/l) over the rearing period of 35 days gave higher increase in survival rate of fry (91.7%) than low concentration of EDTA (0.1g/l) over the rearing period of 35 days (90.8%) compared with the control (88.1%).

II- Length-Weight relationship of *O. niloticus* fry:

Results of length weight relationship of *O. niloticus* fry reared in glass aquaria without EDTA as a control are given in Figure (1). Data showed that, the weight of the fish increases gradually with the increasing length of the fish. The values of (a & b) were -0.0255 and 2.77, respectively. Thus, the length-weight relationship of control group is expressed by the following equation: $W=0.0255\ L^{2.77}$

From the above-mentioned equation, it is clear that, the growth of fish is negatively allometric. The b value is less than the ideal. The correlation coefficient (r) is statistically highly significant (0.98).

The results of length weight relationship of *O. niloticus* fry reared in glass aquaria with 0.1 g EDTA/l are given in Figure (1). Data showed that, the weight of the fish increases gradually with the increasing length of the fish. The values of a and b were 0.0687 and 2.43 respectively. Thus, the length-weight relationship of this treatment is expressed by the following equation: $W = 0.0687\ L^{2.43}$

From the above-mentioned equation, it is clear that, the growth of fish is negative allometric. The b value is less than the ideal. The correlation coefficient (r) is statistically highly significant (0.994).

Results of length weight relationship of *O. niloticus* fry reared in glass aquaria with 0.3 g EDTA/l are given in Figure (1). Data showed that, the weight of the fish increases gradually with the increasing length of the fish. The values of (a & b) were -0.0157 and 2.92, respectively. Thus, the length-weight relationship of this treatment is expressed by the following equation: $W= 0.0157L^{2.92}$

From the above-mentioned equation, it is clear that, the growth of fish is nearly isometric. The b value is nearly ideal. The correlation coefficient (r) is statistically highly significant (0.99).

III- Condition factors of *O. niloticus* fry:

Data in Table (3) revealed that, the composite coefficient of condition (k) and the relative condition factor (kn) varied with the fish size.

The coefficient of condition (k) value of fish fry exposed to different concentrations of EDTA varies from 1.1 to 2.2 with an average of 1.5. It was noticed that, the highest values of condition factor (k) was recorded at small fish size and the lowest occurred at large fish size.

The values of relative condition factors (kn) of fish fry exposed to different concentrations of EDTA ranged between 0.8 to1.7 with an average of 1.1 ± 0.2 in the fish control, 1 ± 0.2 4 in the fish fry exposed to 0.1 g EDTA/l and 1.16 ± 0.25 in the fish fry exposed to 0.3 g EDTA/l.

IV- Growth performance of *O. niloticus* fry:

Data showed that, the total weight gain of *O. niloticus*, fry improved with the high concentration of EDTA compared with the low concentration of EDTA and control; being 406.6 mg in the control and 317.2 mg & 510.4 mg in the fish exposed to 0.1 & 0.3 g EDTA/l

respectively. The highest value of total weight gain (510.4 mg) was detected in the fish exposed to 0.3 g EDTA/l. The same trend was observed with average daily weight gain and specific growth rate (Table 4).

The feed intake (g diet/fish) value for the fish exposed to 0.3 g EDTA/l (337 mg), the value (322 mg) for the fish exposed to 0.1 g EDTA/l and the value of control (325 mg).

The best feed conversion ratio (0.66) was recorded in the fish exposed to 0.3 g EDTA/l compared with the control and fish exposed to 0.1 g EDTA/l (Table 4).

DISCUSSION

Tilapia, are considered as the best species for culture, because of their high tolerance to adverse environmental conditions, ease of reproduction, their fast growth and potential for domestication[25].

In the present study, the fish fry exposed to 0.3 g EDTA /l accelerated larval growth in length, growth in weight and higher survival rate. No abnormalities were observed in fish fry exposed to 0.3 g EDTA /l. Similar reports showed that, EDTA improve survival rates and accelerate growth and development in teleost fry[26-29]. Chelators such as EDTA have been reported to possess antibacterial activity because of the ability of these chelating agents to disrupt bacterial cell membranes by removing essential divalent cations required to link lipopolysaccharide molecules in the outer membrane[30]. Furthermore, strong chelates may inhibit microbial metabolism by binding trace mineral required for cellular reproduction, growth and survival[31].

The relationship between length and weight of the fish is one of the most biological characters of fishes. The weight of the fish increases as a function of its length. Variations in exponent "b" values of fish at various localities may be attributed to variations in the environmental conditions and localities[32-36].

In the present study, the b value was nearly ideal in the fish fry when the fish treated by 0.3 g EDTA /l. Similar observations were noticed for the same species at Lake Qarun[37] (2.9); at Lake Edku[38] (2.98); at culture ponds (nearly isometric)[13] and at laboratory conditions (nearly isometric)[14]. Aleen[39] reported that, the ideal "b" value is 3. However, Lecren[40] reported that, the fish in which the value of "b" ranges between 2.5 to 4 live also in good conditions. In addition, the mean relative condition factors are equal one, which indicates also that, the fish fry exposed to different concentrations of EDTA at laboratory are nearly suitable for the growth of these fish.

The growth promoters improve the immunity, productivity and economic efficiency of fish via its improvement body weight of the fish[41], weight gain[42], feed conversion ratio and efficiency[43].

The present study revealed that, the average values of final body weight, total weight gain and average daily gain (ADG g/fish/day) increases in the fish fry when the fish treated by 0.3 g EDTA /l compared to control. Similar results were also obtained by many authors[26-28]. Tonsy and Abdel-Rahman[29] mentioned that, growth performance in *O. niloticus* was improved due to EDTA application and the effects were more pronounced at 1.5% EDTA/ kg diet, which are considered as the optimum dose that improve the health status and growth parameters of fish. Lead (Pb) and cadmium (Cd) concentrations were significantly decreased in all EDTA treatments in the whole body and fish flesh of Galilee Tilapia fingerlings.

In the present study, the best feed conversion ratio was recorded in fish fry (0.66) when the fish treated by 0.3 g EDTA /l compared to control (0.8). This result was higher than that

recorded in the same species at other farms; being 1.8 at Serow Fish-Farm and 1.21 at fresh water fish farm at Manzalah region[44-45]. It may be due to the feeding in fish fry depend on phyto and zooplankton with artificial food. Culture assays indicate that, both EDTA and EDTA-chelated trace metals enhance the growth of phytoplankton in sea water[46].

Two mechanisms have been proposed for the enhancement of algal growth by chelating agents such as EDTA, which increases the solubility and thus the availability of trace metals that are necessary for growth. However, an alternative hypothesis is that chelating agents reduce the availability of harm metals to phytoplankton by lowering the concentration of harm free metal ions[46,47].

In conclusion, the growth performance of Nile tilapia, *Oreochromis niloticus* fry are good pattern in the fry exposed to 0.3 g EDTA /l. These observations suggest that, the exogenous treatment (0.3 g EDTA /l.) may have practical utility in the fish culture.

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Table (1): Total length (mean ± SD) mm, and total weight (mean±SD) mg, of *O. niloticus* fry at different periods (days).

Days	Total length (mm)			Total weight (mg)		
	Control	0.1 g EDTA/l	0.1 g EDTA/l	Control	0.1 g EDTA/l	0.1 g EDTA/l
0	8.0 ±0 .0	8.0 ± 0.0	8.0 ± 0.0	11.3±0.1	11.3±0.1	11.3±0.1
7	11.3 ± 1.3	10.2 ± 1.0	11.2 ± 1.8	25.6±8.4	17.8±6.0	19.7±9.7
14	15 ± 1.3	15.6 ± 1.6	15.4 ± 2.1	40.3±11.2	41.4±12.3	44.2±18.5
21	20.1 ± 2.3	20.2 ± 1.6	20.5 ± 4.9	101.4±42.0	93.9±23.6	118.8±81.6
28	25.9 ± 4.0	24.1 ± 3.73	26.6 ± 2.5	225.5±89.5	195.0±103.6	254.0±66.4
35	32.3 ± 2.6	29.8 ± 3.2	35.2 ± 3.2	417.9±83.9	328.5 ±106.0	512.7±146.2

Table (2): Survival rate (%) of *O. niloticus* fry, after different periods (days).

Days	Control	0.1 g EDTA/l	0.1 g EDTA/l
0	100	100	100
7	89.4	92	96.6
14	88.1	90.8	91.7
21	88.1	90.8	91.7
28	88.1	90.8	91.7
35	88.1	90.8	91.7

Table (3): Effect of different concentrations of EDTA on the condition factors of *O. niloticus*, reared in glass aquaria.

Days	Control		0.1 g EDTA/l		0.1 g EDTA/l	
	K	Kn	K	Kn	K	Kn
0	2.2	1.4	2.2	1.1	2.2	1.7
7	1.8	1.2	1.7	0.9	1.4	1.1
14	1.2	0.9	1.1	0.8	1.2	1.0
21	1.3	1.0	1.1	0.9	1.4	1.1
28	1.3	1.1	1.4	1.2	1.3	1.1
35	1.2	1.1	1.2	1.3	1.2	1.0

Table (4): Growth performance of *O. Niloticus* fry, reared in glass aquaria with different concentrations of EDTA.

Items	Control	0.1 g EDTA/l	0.1 g EDTA/l
Initial live weight (mg/fish)	11.3± 0.1	11.3± 0.1	11.3± 0.1
Final live weight (mg/fish)	417.9±83.9	328.5±106	521.7±146.2
Weight gain (mg/fish)	406.6	317.2	510.4
Average daily gain (mg/day/fish)	11.6	9.1	14.6
Specific growth rate (% /day)	10.3	9.6	10.9
Feed intake (mg/fish)	325	322	337
Feed conversion ratio (mg/feed/fish)	0.80	1.02	0.66

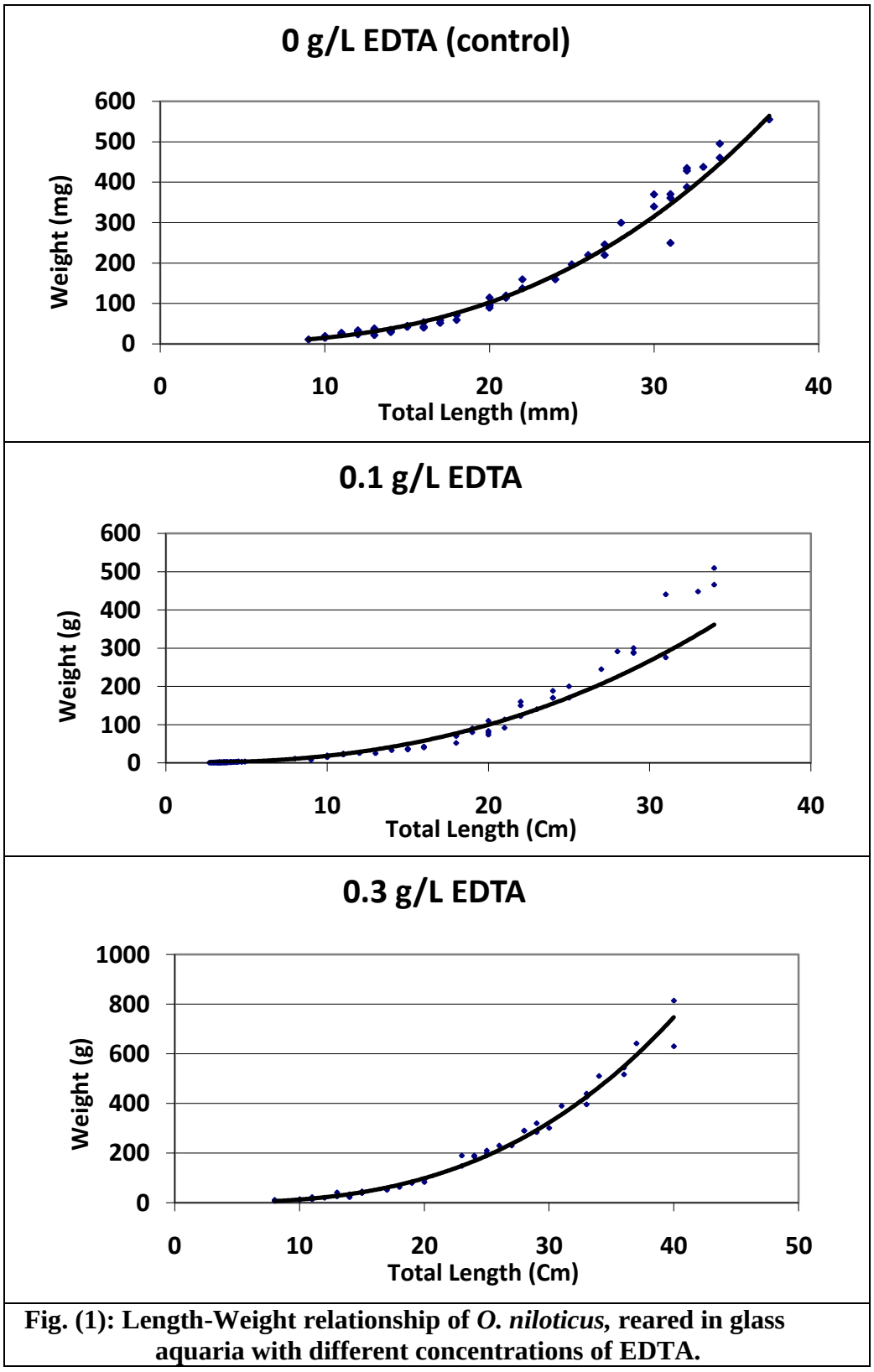

Fig. (1): Length-Weight relationship of *O. niloticus*, reared in glass aquaria with different concentrations of EDTA.

تأثير ايثيلين ثنائى الأمين رابع حامض الخليك (ايدتا) على نمو وأداء زريعة البلطى النيلى (أوريوكرومس نيلوتيكس)

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المستخلص

من اولي المشاكل الخطيرة والهامة في كثير من انحاء العالم هي نقص البروتين الحيواني اللازم للغذاء وسد الفجوة بين الكميات المطلوبة والمنتجة من البروتين الحيواني التي تتزايد عاما بعد عام، والسبب في ذلك هو تزايد عدد السكان. لذا إتجهت جهود الدولة نحو الإرتقاء بقطاع الإستزراع السمكى.

وحيث أن تحفيز نمو وأداء الأسماك فى المزارع السمكية هو الشغل الشاغل لكثير من الباحثين، لذا فان هذا العمل يتناول دراسة تأثير التركيزات المختلفة لمادة ايثيلين ثنائى الأمين رابع حامض الخليك (المجموعه الضابطه، 1و. جم/ لتر، 3و. جم/ لتر) على النمو فى الطول والوزن، معدل البقاء، علاقة الطول والوزن، معامل الحالة وأداء النمو لزريعة البلطى النيلى والمرباة فى أحواض زجاجية.

بينت نتائج الدراسة أن مجموعة الأسماك المعرضة لتركيز 3وجم/ لتر أعطت زيادة فى الطول، الوزن ومعدل البقاء مقارنة بالتركيز الأقل (1و جم/ لتر) والمجموعة الضابطة. بينت نتائج الدراسة أن نمومجموعة الأسماك المعرضة لتركيز 3وجم/ لتر كان متساوى القياس تقريبا (ن=92و2) ومعامل الإرتباط أقوى ما يمكن (99و). بينما كان النمو متفاوت القياس من النوع السالب فى مجموعة الأسماك المعرضة التركيز الأقل (43و2) والمجموعة الضابطة (77و2) ومعامل الإرتباط قوى.

أوضحت نتائج الدراسة أنه لا توجد اختلافات في قيم معامل الحالة، بينما توجد اختلافات طفيفة فى قيم معامل الحالة النسبي لمجاميع الأسماك المعرضة للتركيزات المختلفة. سجلت أعلى القيم في معدل زيادة الوزن، الزيادة اليومية في الوزن ومعدل النمو الخاص في مجموعة الأسماك المعرضة للتركيز الأعلى (3وجم/ لتر) مقارنة بالتركيز الأقل (1و جم/ لتر) والمجموعة الضابطة. بينت الدراسة ان اعلي نسبة تحول غذائي (0,66) فى مجموعة الأسماك المعرضة للتركيز الأعلى مقارنة بالتركيز الأقل (02و1) والمجموعة الضابطة (8 و0).

ونخلص من هذا البحث الي ان نمو وأداء زريعة البلطى النيلى يكون أفضل عند تعرض الأسماك لتركيز 3وجم/ لتر من ايثيلين ثنائى الأمين رابع حامض الخليك (ايدتا).