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Can intraspecific variations be exploited to develop salinity tolerance of *Oreochromis niloticus*?

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ABSTRACT
Intraspecific variation of *Oreochromis niloticus* (Nile tilapia) to salinity tolerance was used through adaptation, selectivity to obtain the optimal *O. niloticus* tolerates salinity in which reproduction, growth, and metabolic rates will not be significantly affected. Three experiments have been conducted; in the first, the salinity tolerant individuals were selected and the survival rate was determined in different salinity degrees from 6 to 36ppt. In the second experiment, the selected parents, (from the first experiment) were put in serial concentrations of salinity from 6ppt (as control) to 26ppt to determine their spawning ability. In the third experiment, fries of selected parents were reared in different salinity degrees, 6ppt (as control), 12ppt, 16ppt and 20ppt. The rearing period of fries of *O. niloticus* continued for 84 days. Chemical body composition of the fish and metabolic efficiency were determined at the end of rearing period. There was increase in the mortality ratio by increasing the salinity. Reproduction efficiency decreased with increasing salinity. The fish chemical body composition showed increasing in dry matter, ether and ash while crude protein decreased by increasing salinity. The growth performance increased gradually with increasing salinity concentration from 6 to 16ppt. Optimum salinity of weight gain in *O. niloticus* was at 16ppt, while 20ppt concentration was the lowest weight gain. In conclusion, the brackish water at 16ppt can be used for rearing *O. niloticus* without affecting growth rate and reproduction with adequate balanced nutrition.

INTRODUCTION

Aquaculture is considered an important available option for reducing the gap between fish production and consumption in Egypt (FAO, 2010). Nile tilapia (*Oreochromis niloticus*) is important freshwater species that are successfully farmed in Egypt with high production rate. *O. niloticus* are herbivorous feeders, which make them a source of clean and healthy animal protein. They are edible fish with high protein content and characterized by their rapid growth rate and possibility of culturing with shrimp in polyculture system. Although *O. niloticus* have fast growth rate but it does not tolerate high salinity.

In the future, due to moving of the world weather towards dryness (greenhouse effect), the consumption of freshwater in agriculture, and increase in the cultivated area in desert at EL-Arish and Rafah and other urban activities, freshwater available for aquaculture will be limited.

These reasons will lead to a serious decline in freshwater. Fresh water is the preferred environment for Tilapia production and may lead to disappear of some Tilapia species. Thus creating a new strain for Tilapia that suits elevated salinities in zones with abundant brackish and saltwater resources, as well as in some arid regions where freshwater resources are limited is crucial (Watanabe *et al.*, 1997).

Salt tolerance is a term that describes the total fitness or fish productivity in a saline environment. The control of salt and water balance in a narrow limit is crucial to life in all multicellular organisms including teleost fish. Salinity tolerance in fish is a mixture of different quantitative traits, such as growth, metabolism, osmoregulation, immune competence and fecundity (Stickney, 1986; Sakamoto and McCormick, 2006; Mancera and McCormick, 2007; Cnaani and Hulata, 2011). Several protocols have been applied to produce salinity tolerant tilapia such as (1) Acclimation (multistep acclimatization, gradual increase or stepwise increase) (2) Selective breeding tolerance to utilize the “additive genetic variability” in a population, by choosing the “best” individuals as breeders (3) Select salinity-tolerant species and developing it by using inter-specific variation in salinity (4) Finally, modification in nutrition such as supplementary dietary with NaCl (Fontaínhas-Fernandes *et al.*, 2000; Ngo *et al.*,2016 and Chourasia *et al.*,2018).

Tilapia species can be cultivated in brackish and seawater environments after acclimatization (Dominguez *et al.*, 2004). Tilapia species tolerate high salinity degrees after gradual transferring from freshwater to salt water (7 up to 70 ppt) (Potts *et al.,* 1967). The fish require pre-acclimation regime and optimal time for acclimation before transferring to full strength seawater. *Oreochromis aureus* and *Oreochromis mossambicus* have showed faster acclimation and better rates of survival in full strength seawater compared with *O. niloticus* (Kamal and Mair, 2005). Prolonged acclimation over days or even weeks from freshwater to salt water also increases survival (Al Asgah, 1984).

The *O. niloticus* exhibits survival until 60 days as moderate tolerance to salinity by direct transfer up to 25 ppt (Watanabe *et al.*, 1985a), but its highest growth is achieved at 0-10 ppt (Villegas, 1990).

The salt tolerance depends basically upon tilapia species, strains, size, adaptation time and environmental factors. Environmental factors include salinity, temperature, dissolved oxygen, ammonia and nitrites levels in water, pH, photoperiod and water turbidity (Lin *et al., 2*000). It was reported that impacts of salinity tolerance within tilapia species or varieties is more affected by size rather than age (Watanabe *et al.*, 1985a), however age also influences tolerance during abrupt salinity changes (Watanabe *et al.*, 1990). Adult fish of red tilapia (*O. mossambicus* × *O. urolepis hornorum*) were more salt tolerant than fry and juveniles. Adult fish tolerated direct transfer to 27 ppt but at 73 ppt, the mortality rate was 100%. Fry and juveniles tolerated direct transfer to 19 ppt without stress and mortality but at 27 ppt, the mortality was 100% (Watanabe *et al.*, 1990). Metabolic rate in tilapia increased with increasing salinity, accordingly energy requirement increased and feed consumption and utilization were affected (Zikos *et al.*,2014). In different salinities marine or freshwater fish, spend more energy to hold sodium and chloride ions in their bodies or take them off (Küçük *et al.*, 2013).

The main goal of this study is to investigate the possibility of using intra specific variation of *O. niloticus* through adaptation, selection, to obtain the optimal strain that can tolerate salinity in which reproduction, growth, and metabolic rates will not be significantly affected.

MATERIALS AND METHODS

The current research is three successive experiments, the results of each experiment leads to the next one. The duration of the three experiments started from October 2014 until October 2015.

1- Mass selection of parents

Fishes were collected from the northern zone of Lake Burullus, where affected by seawater of Mediterranean Sea nearby El-Borg outlet. The salinity degrees in this zone ranged from 6 ppt to 15 ppt. Selecting of the salinity tolerant individuals and determining the survival rates of *O. niloticus* in different salinities were performed through nearly six months, where, these fish were reared in several salinity concentrations. The salinity from 6 ppt to 36 ppt (as seawater) in concrete ponds (2.5 X 5 m, with 1 m depth). Rising of salinity was done by mixing of decholornated tap water with underground saline water. The salinity was increased with ratio 1.0 ppt per week.

2- Testing inbreeding under salinity gradients

The selected parents (from the first experiment) were put in serial concentrations of salinity from 6 ppt (as control) to 26 ppt to determine their ability on spawning in different salinity degrees. Intra-breeding was started on April 2015 and continued for three months. Replicate of three haps (1 m^3) were fixed in concrete ponds and used for each salinity concentration 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26ppt. One male about 20.2 cm total lengths and 145 gram were selected for mating with one female about 16.5cm total length and 115 gram. The measuring of reproduction efficiency depended on the number of resulting fry of each crossing after one month.

3- Fry rearing under different salinities

The fries from intra-breeding were reared in different salinity degrees, 6 ppt (as control), 12 ppt, 16 ppt and 20 ppt. Three replicates for fry rearing under each treatment, 10 fries per hapa. The rearing period of fries of *O. niloticus* continued for 84 days (around three months). The biochemical composition of the diet was (30% protein, 9.7% moisture, 13 ether extract, 6.7% ash and 40.57% carbohydrates and fibers). Feeding ration, one time per day amounted to 4% of total body weight. Water analysis during the experiment period was given in Table (1).The fish chemical body composition and metabolic efficiency was determined at the end of rearing period, according to Association of official analytical chemists (AOAC, 1990).

Table 1: Water chemical analysis in different salinities. Values represent means and standard error.

Parameters	6ppt	12ppt	16ppt	20ppt
Temp.	28.23±0.31	28.22±0.33	28.25±0.33	28.30±0.35
PH	8.350±0.034	8.433±0.033	8.400±0.037	8.367±0.033
DO	7.000±0.139	7.117±0.135	7.150±0.099	7.167±0.156
Amm.	0.257±0.003	0.267±0.003	0.275±0.004	0.282±0.003
NO_2	0.063±0.002	0.067±0.003	0.078±0.003	0.082±0.003

∗ Temp = temperature, DO =dissolved oxygen, Amm= ammonia, NO_2 = nitrate.

RESULTS

Mass selection and survival rate experiment

The survival rate of the adult *O. niloticus* was estimated after acclimation of *O. niloticus* for 2 weeks, in each salinity concentration. There was increase in the mortality ratio by increasing the salinity concentration, so the mortality ratio (died no./initial no.) was 3.7% at salinity 6 ppt, 49.6% at salinity 24 ppt, and 74.4% at salinity 36 ppt Table (2).

Table 2: Initial number, survival and mortality rate of *Oreochromis niloticus* lived in different Salinity degrees that ranged from 6ppt to 36ppt.

Salinity (ppt)	**Period**	**Initial number**	**Survivor Number**	**Survival Rate (%)**	**Dead Number**	**Mortality Rate (%)**
6	Control	163	157	96 .3	6	3.7
12	0.5 month	549	506	92 .2	43	7.8
14	1 month	528	470	89	58	11
16	1.5 month	533	429	80 .5	104	19 .5
18	2 month	553	415	75	138	25
20	2.5 month	600	416	69 .3	184	30 .7
22	3 month	123	74	60.2	49	39.8
24	3.5 month	119	60	50.4	59	49.6
26	4 month	123	55	44.3	68	55.3
28	4.5 month	119	47	39.5	68	60.5
30	5 month	126	43	34.1	83	65.9
32	5.5 month	127	39	30.7	88	69.3
34	6 month	117	33	28.2	84	77.3
36	6.5 month	117	30	25.6	87	74.4

Reproduction efficiency under inbreeding experiment

The number of resulting fry of each cross after one month was used as measurement of the reproduction efficiency. It is noticeable that the number of resulting fry (reproduction efficiency) decreased with increasing salinity. The highest number of fries 245 was at salinity 6ppt and the lowest number of fries, 45 was at salinity 26ppt Table (3).

Table 3: Fry of *Oreochromis niloticus* resulting from crossing one male with one female in different salinities. Values represent means and standard error.

Salinity(ppt)	**Hapa1**	**Hapa2**	**Hapa3**	**Means ±SE**
6	260	246	251	252±4.1
8	235	250	243	243±4.3
10	240	223	230	231±4.9
12	225	210	200	212±7.3
14	185	200	190	192±4.4
16	180	183	170	178±3.9
18	130	145	150	142±6.02
20	120	130	110	120±5.8
22	80	70	65	72±4.4
24	50	60	65	58±4.4
26	40	60	35	45±7.64

Analysis of body composition and Growth performance

There were noticeable differences in fish dry mater composition at salinity 20ppt compared with salinity 6, 12 and16ppt while the lowest dry matter was 27.16 at 16ppt. Crude protein was determined at 6ppt and 12ppt, which were significantly affected compared with salinity concentrations 16ppt and 20ppt. The highest crude protein value was 61.29 at 6ppt and the lowest value was 55.55 at 20ppt Table (4). The ether extract was significantly different at 16ppt and 20ppt in comparison with 6ppt and 12ppt. The highest value was 28.60 at 20ppt and lowest one was 23.75 at 6ppt Table (4). In ash, the concentration 12ppt showed significant difference compared with 6, 16, and 20ppt and the highest value was at 20ppt Table (4).

Table 4: Chemical body composition of *Oreochromis niloticus* at different salinities at 84 days. Values represent means and standard error. Superscripts represent significantly different results.

Parameters	Salinity levels			
	6ppt	12ppt	16ppt	20ppt
Dry matter	27.30±0.07[b]	27.29±0.04[b]	27.16±0.05[b]	27.91±0.02[a]
Crude protein	61.29±0.69[a]	58.99±0.22[a]	57.29±0.51[b]	55.55±0.12[c]
Ether extract	23.75±0.59[b]	24.56±0.18[b]	27.59±0.43[a]	28.60±0.28[a]
Ash	14.25±0.08[c]	15.52±0.21[a]	14.19±0.07[c]	14.84±0.10[b]

The growth performance was estimated after 84 days. Results of SGR (specific growth rate) showed significant differences between treatment one (6ppt) compared to treatments three and four (16ppt and 20ppt).The highest value of SGR was1.67 in treatment no.1, under salinity 6ppt, while the lowest value was 0.66 in treatment no. 4 salinity 20ppt. Average daily gain, ADG showed significant differences between treatment no.1 and 4 compared to treatment no. 2 and 3 (table 5), where the highest value was 1.24 in treatment no. 3 and the lowest value was 0.69 in treatment no. 4 Table (5).

Table 5: Growth performance of *Oreochromis niloticus* at different salinities at 84 days. Values represent means and standard error. Superscripts represent significantly different results.

Parameters	Salinity levels			
	6ppt	12ppt	16ppt	20ppt
Initial weight	10.83±1.30[c]	15.11±1.92[b]	21.67±0.96[a]	25.67±0.38[a]
Final weight	83.64±6.94[b]	115.06±3.35[a]	128.09±7.36[a]	84.87±4.21[b]
Weight gain	72.81±6.18[b]	99.95±3.22[a]	106.42±8.07a	59.20±4.38[b]
SGR	1.67±0.11[a]	1.61±0.14[ab]	1.28±0.10[b]	0.66±0.05[c]
ADG	0.85±0.07[b]	1.16±0.03[a]	1.24±0.09[a]	0.69±0.05[b]

* SGR = specific growth rate, **A**DG = Average day gain.

DISCUSSION

Many species of tilapia are euryhaline; tolerate wide range of salinity, mainly because they are developed from a marine teleost ancestry (Suresh and Lin, 1992).

In the current study, the acclimation of Nile tilapia (*O. niloticus*) from freshwater to salt water achieved gradually from 6ppt to 36ppt. The survival rate of acclimated fish in saline water was significantly high (80.5% - 96.3%) at16ppt, while, it was low (30% -40%) at 28ppt. When fishes are subjected to elevated salinity levels, chloride cells in the gill filaments proliferate and Na+/ K+ ATPase activity increase to regulate salt concentration in blood to suite the elevated salinity (Avella and Doudet, 1993).

In the present study, 18ppt is considered the MST (mean salinity tolerance) since the mortality was about 25%, while, the mortality of the fry resulting from inbreeding of *O.nilotcus* that were reared in the 18ppt was 50%.

Early studies with tilapia described salinity tolerance (ST) as the highest salinity before death (Chervinski and Yashouv, 1971) while the incipient salinity tolerance (IM) is the cumulative survival (CS100) and it is defined as the level at which a variety initially exhibits mortality associated with increasing salinity. Similarly, commercially incipient mortality level is defined as the salinity at which a variety reaches 15% mortality (CS85) and lethal salinity is defined as the salinity at which a variety suffers complete mortality (CS0). In the current study, 6ppt is considered the IM while salinity more than 36ppt is considered the CS0 since mortality is more than 75%.

Effects of increasing salinity in tilapia can cause sub-lethal end points where the behavior is disrupted. Behavior disruption includes low performance reproduction, increased incidence of disease, cessation of feeding, disease development, sluggishness with rapid pectoral and opercular movements, erratic swimming behavior, decreased weight gain and failure to respond to gentle touch (Altinok and Grizzle, 2001).

The results of the current research agree with Küçük *et al.* (2013) who reported that *O. niloticus* acclimated to 8ppt of salinity and transferred to four different salinities (12, 16, 20 and 24ppt) had no significant difference among the first three treatments while decreased growth rate was observed at 24ppt. Similarly, our results agree with Stickney, (1986) who found that *O. niloticus* survived after direct transferring to 50% seawater (17ppt), but not 75% (26ppt) seawater.

Water salinity has also been reported to affect reproduction of tilapia. The total number of Nile tilapia spawning females is greater in brackish water (5-15ppt) than in either full strength seawater (32ppt) or freshwater (Watanabe e*t al.*, 1985 a).In the present study the fry production decreased at high salinity.

The decrease was 50% lower at 20ppt than at 6ppt. Fineman-Kalio, (1988) reported that gonadal development and spawning of tilapia occurred at salinities 17-29ppt, while the start of reproduction was retarded with increasing water salinities 25-50ppt and reproduction stopped completely at salinity above 30ppt. High salinity seems to delay gonadal development in *O. aureus* and *O. niloticus* (Fineman-Kalio, 1988). Egg and fry production per body weight in fingerlings decreased with salinities above 18ppt (Watanabe *et al.*, 1989).

The growth inhibition at high salinity on freshwater fish can be explained by the higher energy cost for osmoregulation (Prunet and Bornancin, 1989). Salinities for optimum growth of Nile tilapia ranged from 5 to 10ppt (Payne, 1983). *O. niloticus* survived only for brief periods in full strength seawater (Cataldi *et al.*, 1988).

In the present study optimum salinity of weight gain in *O. niloticus* was at 16ppt salinity concentration, while 20ppt concentration was the lowest weight gain. Early exposure to salinity through spawning and hatching under elevated salinities improves salinity tolerance of young tilapia and may facilitate acclimation to seawater (Watanabe *et al.*, 1985b). Pre acclimation during spawning and hatching has a crucial physiological significance for some euryhaline teleost during seawater adaptation. However, osmoregulation mechanism is energy demanding processes, species with low metabolic rates utilizing about 20 to 50% of the overall energy available for salinity adaptation (Ramos *et al.,* 2010).

In the current study, the growth performance increased gradually with increasing salinity concentration from 6ppt to 16ppt, where ionic gradients between the blood and the water are minimum and this saves energy that will accordingly directed towards growth. While the growth performance decreased at 20ppt salinity concentration, because more energy was utilized to regulate the gradient concentration of that salinity than conserved for growth.

The high metabolic rates at higher water salinities apparently reflected a significance decline in growth rates. High metabolic rates are highly correlated with decreased protein content and increased ethyl ether concentration (Morgan and Iwama, 1991). This means that at high salinity the fish utilize protein to produce the essential energy for metabolic process. Altinok and Gizzle (2001) reported that the needs of nutrients and protein may differ between freshwater and salt water species to influence metabolism and homeostatic processes in fish. Also, high metabolic rates can be explained by action of salinity upon digestive enzymes, where the exposure to different salinities change the ionic concentration in the gastric lumen, consequently may alter digestive enzymes activities which affect feed digestibility and fish growth performance (Vargas-Chacoff *et al.*, 2015).

Moreover, previous studies suggested that decreasing fish growth in very low or high salinities has potential to suppress the appetite where it is related to the decrease in food consumption with high-energy demand for osmoregulation (Mahmudul *et al.,* 2014). Jones and Clemmons, (1995) found in salmoned that oxygen consumption rates were low in freshwater, not in isotonic salinity. In the present study the water analysis indicated that no effect of salinity concentration on water components and consequently the growth gain was due to salinity concentration only.

Tilapia has been observed to grow better in brackish water than fresh water. Woo *et al.* (1997) found that *O. niloticus* kept in 15ppt had higher growth rates than fish kept in 0 ppt (fresh water). Similarly, (Ninh *et al.*, 2014) reported that growth performance of *O. niloticus* was enhanced in brackish water (15-20ppt). Also, Iqbal *et al.* (2012) found that *O. niloticus* in higher salinities had higher growth rate than freshwater.

Azevedo *et al.* (2015) examined the effect of water salinity on daily weight gain in 45 days experiment and exposed *O. niloticus* to different salinities (0, 7, 14 and $21gL^{-1}$). The best results were observed for the water salinity 0 and 7 gL^{-1} while increasing salinities caused decreased weight. Vonck *et al.* (1998) reported high growth rate in *O. niloticus* was at 8 to 24ppt and (Kang'ombe and Brown, 2008) found that growth increased in *Tilapia rendalli* at 5 to 10ppt.

In conclusion, the brackish water at 16ppt can be used for rearing *O. niloticus* without affecting growth rate and reproduction with adequate balanced nutrition. Salinity tolerance for *O. niloticus* is crucial to produce salinity resistant hybrids to overcome freshwater shortage for fish aquaculture.

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ARABIC SUMMARY

هل يمكن إستغلال الإختلافات الوراثيه بين الأفراد في تحسين مقاومة أسماك البلطي النيلي للملوحه ؟

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تم إستغلال الإختلافات الوراثية بين أفراد أسماك البلطى النيلى لتحسين صفة تحمل الملوحة من خلال عملية الأقلمة والإنتخاب، لذالك تم إجراء ثلاثة تجارب:
التجربة الأولى: تم إنتخاب الأفراد المقاومة للملوحة فى مدى من ٦ إلى ٣٦ جزء فى الألف.
التجربة الثانية: تم إنتخاب الآباء الناتجة من التجربة الأولى وإختبار قدرتها على إنتاج النسل فى تركيزات ملوحة مختلفة من ٦ إلى ٢٦ جزء فى الألف.
التجربة الثالثة: تم تربية اليرقات الناتجة من الآباء (فى التجربة الثانية) وتعريضها لدرجات ملوحة من ٦ إلى ٢٠ جزء فى الألف واستمرت فترة التربية حتى ٨٤ يوم.
فى نهاية فترة التجربة تم دراسة التركيب البيوكيميائى للأسماك،كذالك كفاءة التمثيل الغذائى.
أوضحت النتائج:

- زيادة في معدلات موت الأسماك بينما قل عدد اليرقات مع زيادة الملوحة.
- أوضح التركيب البيوكيميائى للأسماك زيادة فى نسبة المادة الجافة والإثير والرماد بينما قلت نسبة البروتين الخام بزيادة الملوحة.
- حدوث زيادة فى معدلات النمو فى التركيزات من ٦ إلى١٦ جزء فى الألف وكانت درجة الملوحة المثلى لحدوث زيادة فى الوزن عند ١٦ جزء فى الألف.

مما سبق يتضح ان درجة ملوحة ١٦ جزء فى الألف هى أنسب ملوحة لنمو البلطى النيلى وتكاثره وذلك مع وجود تغذية متوازنة.