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Rearing of the thin-lipped mullet, *Liza ramada*, broodstock in treated groundwater

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ABSTRACT

Saline groundwater is the substitute for seawater; especially when it is not possible to establish the hatcheries near the shore. The aim of this study is to assess the suitability of untreated or treated saline groundwater for the survival, growth and reproduction of *L. ramada* broodstock. The treatment of saline underground water was carried out in three steps: mechanical filtration, chemical treatment with chlorine and biological treatment with green algae; filamentous algae and *Nannochloropsis occulata*. Immature mullet broodstock was stocked in different waters; treated and untreated saline groundwater, for six months to follow up on growth performance and reproductive activity in these waters. High levels of some elements such as iron and manganese as well as hydrogen sulfide gas and high levels of ammonia were observed in the untreated saline groundwater in comparison to those of treated water. The first step of water treatment is the mechanical stage, where the water is filtered mechanically, in which hydrogen sulfide gas is disposed of, then the second chemical stage uses chlorine, which oxidized and precipitated both iron and manganese, followed by the third biological stage, in which green algae; filamentous algae and *N. occulata* are used, which work to eliminate the ammonia. The treatment of saline groundwater increased the survival rate and improved the growth of mullet broodstock; since high values of growth in length and weight and better condition factors were recorded for fish stocked in treated water. In addition, the reproductive activity of mullet broodstock was increased in treated water. High values of hepatosomatic index (HSI), gonadosomatic index (GSI), and a high percentage of frequency for maturity gonad stages were obtained in treated saline groundwater. The present study concluded that treatment of saline groundwater is essential for the improvement of water quality, growth performance, and the reproductive activity of mullet broodstock.

INTRODUCTION

The thin-lipped mullet, *Liza ramada* (Mugilidae), is one of the most important fish species farmed in Egypt, has the ability to grow easily in different culture systems (mono & poly culture) and with different densities **(Khalil, 2001** and **Nawareg *et al.*, 2020**). Nevertheless, the culture of *L. ramada* was depending on the collection of its fries from natural fisheries. Thus, production from domestic hatcheries can improve availability and probably reduce the cost of purchasing fry. To substitute the collection of mullet fries from natural habitat, marine hatcheries were the ideal solution for this, but they require capabilities and infrastructure based on location, source and water characteristics, and it will not always be possible to establish them near the shore (**Mousa, 2010** and **Mousa & Khalil, 2013**).

To establish marine hatcheries far away from the see it is important to find out a source of saline water has good quality and suitable for the reproduction of marine fishes. Underground saline water presents the prospect to substitute the sea water and increase the production of euryhaline and marine species (**Allan *et al*., 2009**). Groundwater has been used for cultivation of some species, such as tilapia, sea bream, eels and channel catfish (**Forsberg *et al.,* 1996; Ingram *et al.,* 1996** and **Samocha *et al*., 1998**). In order to achieve the sustainability of natural resources, alternative solutions may be available for mullet fish broodstock through the use of large reserves of saline groundwater. A large amount of saline groundwater was available for aquaculture, but little was known about the pond management, production and reproductive performance of *L. ramada* fish in saline groundwater.

The saline groundwater is rich in nutrients that does not contain undesirable organisms (pathogen free) and it is mostly has a constant temperature (**Singh *et al.,* 2017**). A relative elevation of some elements such as iron and manganese as well as hydrogen sulfide gas and high levels of ammonia were observed in saline groundwater (**Al-Haddad *et al*., 2014** and **Al-Hajeri1 & Amer; 2021**). So the treatment of saline groundwater is essential to improve its quality. The existence of both iron and manganese in groundwater is problematic in water due to their tendency to oxidize and precipitate as indissoluble oxides under a variety of conditions, causing aesthetic and practical problems in water (**McPeak & Aronovitch, 1983**). Therefore, oxidation of soluble iron and manganese is should be followed by filtration using mechanical filtration to eliminate the insoluble oxides of both iron and manganese. Chlorine (Cl^-) is added to water to control harmful bacteria as a disinfectant, it is also considered as an oxidizing agent that capable of oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), usually as iron hydroxide (**Heinen, 1996**). Ferric iron capable of agglutinate to cell membranes and harm the fish gill epithelium and prevent diffusion of ions through cell membranes, and causing changes in plasma steroid concentrations and serum ion regulation (**Lappivaara *et al.,* 1999** and **Teien *et al.,* 2008**). Also, the exposure to high concentrations of ferric iron may damage gills (**Dalzell & Macfarlane, 1999**). Under these conditions, fish not only can't able to survive, but also can't grow or reproduce in waters with iron concentrations >1.0 mg/L (**Sykora *et al.,* 1972; Smith *et al.,* 1973** and **Steffens *et al.,* 1993**). Algae are primary producers capable of using nutrients commonly present in water (e.g., nitrogen and phosphorus) to promote energy conversion (**Zhao *et al.*, 2018** and **Rossi *et al.*, 2020**). Algae cells are also capable of removing metals (**Salama *et al.*, 2019** and **Lin *et al.,* 2020**), sequestrating CO_2 (**Raeesossadati *et al.,* 2014; Hallenbeck *et al.,* 2015**), and removing organic matter in the absence of sunlight (**Celis-Pla *et al.*, 2015**).

The present study aimed to assess the suitability of untreated or treated saline groundwater for survival, growth and reproduction of *L. ramada* broodstock.

MATERIALS AND METHODS

Chemical analysis of saline groundwater:

In the present work, the chemical analysis and treatment of saline water, obtained from an underground well at El-Matareya Research Station, Dakahlia Governorate, National Institute of Oceanography and Fisheries (NIOF), at a depth of (60 meters), were done to investigate its suitability for thin-lipped mullet broodstock rearing, growth and maturation.

Determination of hydrogen sulfide (H_2S):

The concentration of H_2S in saline groundwater was determined by the spectrophotometric method according to **Suresha *et al*. (2008)**.

Determination of ammonia (NH_3):

NH_3 concentration was obtained by ammonia high range portable photometer (HI 96733); using HI 96 series kit.

Analysis of major and minor elements:

Water chemical analysis was performed with Inductively coupled plasma-mass spectrometry (**ICP-MS**): **ICP-MS** is a powerful technique for trace multi element and isotopic analysis, because of its high sensitivity and ability to determine the isotope composition of a sample using fast pretreatment procedures than other mass spectrometry techniques.

Treatment of saline groundwater:

The treatment of saline groundwater was carried out in three steps: mechanical filtration, chemical treatment with chlorine and biological treatment with green algae; filamentous algae and *N. occulata*. The first step is the mechanical stage, where the water is filtered and passed through a mechanical filter, in which hydrogen sulfide gas is disposed of, then comes the second, chemical stage using chlorine at a dose of 200µ*l*/ liter of water, which is an oxidizing agent that oxidizes and precipitates both iron and manganese, then after disappear of chlorine by strong and continuous aeration, the third biological stage in which green algae; filamentous algae and *N. occulata* are used, which work to eliminate ammonia from saline underground water.

Effect of water quality on growth performance and reproductive activity:

Immature mullet broodstock (of both sexes), with standard length larger than 30 cm, were stocked in the different waters; treated and untreated saline groundwater, to follow up the growth performance and the reproductive activity in these waters. Fish were fed at 3% rate of live body weight (BW) twice daily at 9.00 a.m and 16.00 p.m. The experimental treatments were triplicated and lasted for six months (July-December). Mullet broodstock were collected alive monthly during the experiments to study the growth performance and the reproductive activity.

Growth performance:

After the collection of fishes, their total and standard lengths were measured to the nearest 0.1 cm and their weights to the nearest 0.1 g.

Condition factor:

The condition factor (k) for each fish was determined from the following equation:

$$K = W \times 100 / L^3 \qquad \text{(Le-Cren, 1951)}$$

Where W = weight in gram and L = length in cm.

Feed conversion:

The Feed conversion was determined as following:

Feed conversion= (feed given per fish) / (weight gain per fish)

Reproductive activity:

The gonads and liver were extirpated from the body cavity, weighed to the nearest 0.01 g. Hepatosomatic index (HSI) was calculated for each maturity stages according to **Sokal & Rohlf (1969)** as in the following equations:

HSI = (Weight of the liver/ Gutted weight) x 100

Gonadosomatic index (GSI) was calculated for each fish according to the following formula:

GSI = (Weight of the gonad/ Gutted weight) x 100

For the measurements of the oocyte diameter, the oocytes were preserved in a solution of 1% formalin in 0.6% NaCl. They were then placed on a glass slide and measured with an ocular micrometer. The maturity stages were assessed according to **Mousa (1994)**. The assessments were based on the bases of seasonal changes in the histomorphology and gonadosomatic index. The monthly percentage of each maturity stages in males and females were calculated and recorded. Also, the sizes and the percentage of prespawning oocytes were determined in the obtained prespawning females.

Statistical Analysis

Data were analyzed by SPSS (Statistical Package for the Social Sciences, IBM version 22) (**SPSS, 1999**). Statistical significance was accepted at $P < 0.05$.

RESULTS

Treatment and chemical analysis of saline groundwater:

The treatment of saline underground water was carried out in three steps: mechanical filtration, chemical treatment with chlorine and biological treatment with green algae; filamentous algae and *Nannochloropsis occulata*. The chemical analysis of sea water, raw saline groundwater and treated saline groundwater was obtained in **Table (1)**. In general, the salinity of waters was adjusted at the salinity of groundwater of 28‰ to success the comparison of results. The obtained results indicated that the raw groundwater has higher levels of hydrogen sulfide, ammonia, major elements except potassium, and minor elements except arsenic and mercury in comparison with sea water. As indicated in **Table (1)**, the treatment of saline groundwater removed hydrogen sulfide and reduced ammonia to a minimum level in addition to reducing major and minor elements to levels similar to those in seawater.

Table (1): Chemical Analysis of coastal seawater, raw saline groundwater and treated saline groundwater

Item	Coastal seawater diluted* (28‰)	Raw groundwater (28‰)	Treated groundwater (28‰)
pH	**8.9**	**8.37**	**8.74**
EC (ms)	**35.31**	**39.64**	**37.39**
Hydrogen sulfide (H_2S)	**ND**	**5**	**ND**
(mg L^{-1})	**ND**	**30**	**0.005**
Ammonia (NH_3) (mg L^{-1})			
Major elements (mg L^{-1})	**7930.584**	**8251.341**	**8071.268**
Sodium (Na)	**320.871**	**220.324**	**178.376**
Potassiunm (K)	**1040.963**	**1174.598**	**970.310**
Magnesium (Mg)	**1126.131**	**1980.531**	**1698.9**
Calcium (Ca)	**7.945**	**15.122**	**12.144**
Strontium (Sr)	**0.937**	**1.587**	**1.206**
Manganes (Mn)	**7.087**	**7.667**	**5.204**
Boron (B)			
Minor elements (mg L^{-1})	**0.001**	**0.0026**	**0.0018**
Barium (Ba)	**0.012**	**0.020**	**0.017**
Znic (Zn)	**0.0004**	**0.0005**	**0.0001**
Cobalt (Co)	**0.0024**	**0.0029**	**0.002**
Nickel (Ni)	**0.0367**	**0.0512**	**0.049**
Iron (Fe)	**0.0039**	**0.0019**	**0.0004**
Arsenic (As)	**0.0014**	**0.0019**	**0.0009**
Lead (Pb)	**0.161**	**0.184**	**0.079**
Aluminum (Al)	**0.0066**	**0.0098**	**0.0087**
Chromium (Cr)	**0.0016**	**0.003**	**0.0019**
Cupper (Cu)	**0.0019**	**0.0015**	**0.0009**
Mercury (Hg)	**0.0098**	**0.013**	**0.0096**
Gallium (Ga)	**0.00045**	**0.001**	**0.001**
Vanadium (V)			

*: values calculated from coastal seawater at 42‰

Growth performance:

The results of the effect of water quality and its treatment on the growth of *L. ramada* broodstock were summarized and recorded in **Table (2)**. The treatment of saline groundwater improved the growth of mullet broodstock; since high values of growth in length and weight were recorded for fish stocked in treated water. The total gains in weight were 165±1.06 g/fish and 245±1.42 g/fish for male and female respectively **(Table 2)**.

Feed conversion:

The food conversion ratio was 0.56±0.02 for males and 0.50±0.01 for females of mullet broodstock reared in treated saline groundwater as indicated in **Table (2)**.

Condition factor:

The condition factor (K) is a measure of the degree of well being of fish (**g/cm^{-3}**). The obtained results showed that *L. ramada* broodstock reared in treated saline groundwater had better condition factor (1.06±0.05 for females and 0.94±0.03 for males) than that reared in untreated saline groundwater (0.82±0.03 for females and 0.88±0.01 for males) **(Table 2)**.

Survival rate:

The treatment of saline groundwater increased the survival rate of mullet broodstock; since high value of survival (100%) was recorded for fish stocked in treated water **(Table 2)**.

Table (2): Growth performance and reproductive activity of *L. ramada* broodstock reared in raw saline groundwater and treated saline groundwater for six months

Item	Raw underground water		Treated underground water	
	Male	Female	Male	Female
Initial average length (cm/fish)	25±0.22	26.5±0.13	25±0.26	26.5±0.13
Final average length (cm/fish)	30.5±0.16	34.5±0.20	31.5±0.35	33.5±0.39
Initial average weight (g/fish)	130±2.49	155±3.15	130±2.32	155±2.20
Final average weight (g/fish)	251±3.26	335±3.37	295±3.45	400±2.89
Total gain in weight (g/fish)	121±1.02	180±1.15	165±1.06	245±1.42
Food conversion ratio	0.65±0.01	0.58±0.01	0.56±0.02	0.50±0.01
Condition factor	0.88±0.01	0.82±0.03	0.94±0.03	1.06±0.05
Survival rate (%)	75±2.00	70±1.00	100±0.00	100±0.00

Reproductive activity:

The obtained results in **Tables (3-6)** indicated that the reproductive activity of mullet broodstock was increased in treated saline groundwater. High values of both hepatosomatic index (HSI) and gonadosomatic index (GSI), and high percentage of frequency for maturity gonad stages were obtained in treated saline groundwater **(Tables 3-6)**.

Hepatosomatic index (HSI):

The hepatosomatic index increased gradually during the gonad development in both raw and treated waters recorded high values in mullet broodstock reared in treated saline groundwater and gave the highest values of 1.27±0.50 and 2.15±0.25 for ripe males and prespawning females, respectively as indicated in **Tables (3&5)**. However, lower HIS were obtained for mature fishes in raw saline groundwater; 0.96±0.26 and 1.69±0.50 for males and females, respectively **(Tables 3 & 5)**.

Gonadosomatic index (GSI):

Gonadosomatic index (GSI) and hepatosomatic index (HSI) are closely related to each other. In general, they represented higher levels in females than in males of mullet. GSI was recorded higher values during gonad maturation for mullet broodstock reared in treated water (9.6±0.51 for ripe males and 20.4±1.02 for prespawning females) than those of mature fishes in raw water (7.3±0.04 and 14.3±0.12 for males and females respectively) as indicated in **Tables (3 & 5)**.

Table (3): Monthly variations in the frequency (%) of testicular stages of *L. ramada* during testicular cycle in both raw saline groundwater and treated saline groundwater for six months

Month	**Water type**	**Fish no.**	***I***	***II***	***III***	***IV***
Jul	Raw saline groundwater	10	100			
	Treated saline groundwater	10	100			
Aug	Raw saline groundwater	10	100			
	Treated saline groundwater	10	100			
Sep	Raw saline groundwater	10	70	30		
	Treated saline groundwater	10	50	50		
Oct	Raw saline groundwater	10	40	30	30	
	Treated saline groundwater	10	20	30	50	
Nov	Raw saline groundwater	10		50	30	20
	Treated saline groundwater	10		20	40	40
Dec	Raw saline groundwater	10			20	80
	Treated saline groundwater	10				100

Table (4): Gonadosomatic index (GSI%) and hepatosomatic index (HSI%) of males *L. ramada* at different stages of maturation reared in raw saline groundwater and treated saline groundwater for six months

Testis Stage	Water type			
	Raw saline groundwater		Treated saline groundwater	
	GSI%	HIS%	GSI%	HIS%
I	0.40±0.11*	0.90±0.13*	0.45±0.20	1.10±0.215
II	0.65±0.20*	0.92±0.24*	0.70±0.30	1.15±0.144
III	2.10±0.30*	1.10±0.17*	2.80±0.45	1.18±0.254
IV	7.30±0.40*	0.96±0.16*	9.60±0.51	1.27±0.15

*Mean was significantly lower than that of treated saline groundwater ($P < 0.05$).

Table (5): Monthly variations in the frequency (%) of ovarian stages of *L. ramada* during ovarian cycle in both raw saline groundwater and treated saline groundwater for six months

Month	Water type	Fish no.	I	II	III	IV	V
Jul	Raw saline groundwater	10	100				
	Treated saline groundwater	10	100				
Aug	Raw saline groundwater	10	100				
	Treated saline groundwater	10	100				
Sep	Raw saline groundwater	10	80	20			
	Treated saline groundwater	10	70	30			
Oct	Raw saline groundwater	10	50	20	20	10	
	Treated saline groundwater	10	30	30	20	20	
Nov	Raw saline groundwater	10		40	30	20	10
	Treated saline groundwater	10		20	30	30	20
Dec	Raw saline groundwater	10				20	80
	Treated saline groundwater	10					100

Table (6): Gonadosomatic index (GSI%) and hepatosomatic index (HSI%) of females *L. ramada* at different stages of maturation reared in raw saline groundwater and treated saline groundwater for six months

Ovary Stage	Water type			
	Raw saline groundwater		Treated saline groundwater	
	GSI%	HIS%	GSI%	HIS%
I	0.45±0.13*	0.95±0.19**	0.60±0.18	1.52±0.25
II	0.75±0.25*	0.97±0.23**	0.88±0.22	1.69±0.14
III	1.95±0.55**	1.16±0.27**	3.20±1.12	1.82±0.24
IV	4.95±1.85**	1.15±0.13**	10.5±2.15	1.89±0.25
V	14.3±0.12**	1.69±0.50*	20.4±1.02	2.15±0.25

*Mean was significantly lower than that of treated saline groundwater ($P < 0.05$).
**Mean was significantly lower than that of treated saline groundwater ($P < 0.01$).

DISCUSSION

The present study has investigated the suitability of treated saline groundwater for survival, growth and reproduction of *L. ramada* broodstock. Saline groundwater quality has an active and key role in the pond management, production and reproductive performance of marine fish (**Fielder *et al.*, 2001; Jana *et al.*, 2004; Antony *et al.*, 2021 and Kumari *et al.*, 2021**). The improvement of saline groundwater by various treatments is esssential for its suitability for fish rearing (**Jana *et al.*, 2004; Shakeeb-Ur-Rahman *et al.*; 2005; Bhatnagar & Devi, 2013 and Antony *et al.*, 2021**). In the present experiment, treatment of saline underground water was carried out in three steps: mechanical filtration, chemical treatment with chlorine and biological treatment with green algae; filamentous algae and *Nannochloropsis occulata*.

The results of the water analysis showed that the treatment of groundwater by the mentioned methods led to the approximation of the components of groundwater to those produced in sea water. The use of sand filter followed by aeration of groundwater in the current study led to the removal of major part of hydrogen sulfide. Similar results were obtained during the treatment of hydrogen sulfide-rich saline groundwater (**Al-Haddad *et al*., 2014**). The use of chlorine proved effective in getting rid of the remaining part of hydrogen sulfide and precipitating part of the high-concentration elements in the groundwater. Similarly, chlorination or activated carbon adsorption can be utilized as a polishing process to remove the residual hydrogen sulfide, depending on its concentration in the water after the aeration treatment and the rate of groundwater pumping (**Al-Haddad *et al*., 2014**). Finally, using bioremediation with green algae; Filamentous algae and *Nanochloropsis aculata* it was possible to remove ammonia and part of the highly concentrated minerals in the groundwater. The use of algae for water treatment; in ammonia and salt removal from saline water, and the production of water for a variety of purposes, is a new and cost-effective concept (**Gagneux-Moreaux *et al*., 2006; Park *et al*., 2010; Kesaano & Sims, 2014; Moayedi *et al*., 2019; Nagy *et al*., 2019 and Guo *et al*., 2021**).

Water treatment can optimize the application of underground and surface water for fish farming and with continuous monitoring of all parameters, successful aquaculture development can be maintained (**Makhamisi, 2019**). The obtained chemical analysis of water indicated that the treatment of saline groundwater is necessary to improve the water quality so that it is suitable not only for the performance of the survival and growth of the mullet broodstock but also for the increase of their reproductive activity. Similarly, a periphyton-supported aquaculture system can be used successfully for the culture of *M. cephalus* in inland saline groundwaters (**Jana *et al*., 2004**).

Although the mineral profiles differ between saline groundwater and natural seawater, saline groundwater is suitable for the reproduction and growth of *L. ramada* broodstock. The present treatment of saline groundwater improved the growth performance of mullet broodstock. Similar high growth, (SGR and per cent increase in body weight), feed conversion efficiency and intestinal enzyme activity were observed in grey mullet, *M. cephalus*, reared in inland saline groundwater (**Barman *et al*., 2005**). Also, cobia, *Rachycentron canadum*, can achieve optimal growth in treated saline groundwater of low and intermediate salinities (**Antony *et al*., 2021**). In this respect Gulf killifish, *Fundulus grandis*, grew and spawned successfully in saline groundwater (**Phelps *et al*., 2010**). The obtained results of mullet growth in raw saline groundwater; total gain in weight, the food conversion ratio, the condition factor and survival rate were significantly lower than that obtained for fish reared in treated water. The observed survival rate for mullet fish reared in raw saline groundwater ranged from 70% to 75%. On the contrary, complete mortality was reported in Australian snapper, *Pagrus auratus*, (**Fielder *et al*., 2001**), barramundi, *Lates calcarifer* (**Partridge & Creeper, 2004** and **Partridge & Lymbery, 2008**), rabbitfish, *Siganus rivulatus*, and mulloway, *Argyrosomus japonicus* (**Doroudi *et al*., 2006** and **Mourad *et al*., 2012**)***, and*** silver pompano, *Trachinotus blochii* (**Pathak *et al*., 2019**) reared in raw saline groundwater.

The present results indicated that the reproductive activity of mullet broodstock was increased in treated saline groundwater. High values of both hepatosomatic index (HSI) and gonadosomatic index (GSI) and high percentage of frequency for maturity gonad stages were obtained in treated saline groundwater. As a possible explanation of these results, there is a high level of calcium in BRS underground brackish water that causes calcitonin secretion, which positively affects reproduction (**Evans *et al*., 2005** and **Mohammadi *et al*., 2011**). Similarly, the reproductive success of Gulf killifish, *Fundulus grandis*, in inland saline waters was within the range expected for fish reproducing in natural seawater (**Phelps *et al*., 2010**). Also, underground water can be used as a source to induce successful sexual maturation and to produce high-quality gonads in rainbow trout, *Oncorhynchus mykiss* (**Mohammadi *et al.*, 2011**).

CONCLUSION

Overall, the present study suggests that the treatment of saline groundwater can be used successfully to improve its quality and make it suitable for the culture of mullet broodstock and thus could contribute to the development of sound and sustainable aquaculture technology of mullets. Aquaculture of marine species like *L. ramada* in inland areas using saline groundwater can be an alternative to aquaculture in coastal areas, where the availability and cost of land and water may be impossible. Further studies should be oriented on physiological changes in *L. ramada* broodstock reared in saline groundwater under different conditions.

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Conflict of Interest

The author declares there are no conflicts of interest.

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