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Growth, Feed Utilization, Blood Biochemical Variables, Immunity, Histology of the Intestine, Gills and Liver Tissues, and Carcass Composition of the European Seabass (*Dicentrarchus labrax*) Raised Using Different Water Sources

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ABSTRACT

Given the shortage of freshwater, mariculture is a promising method to maintain the development of aquaculture. This study compared the impact of two different groundwater sources of saline water on the productivity and overall health status of juvenile seabass. With the same stocking density, seabass fingerlings with an average body weight of 12.0±0.25 g fish-1 and an average length of 10.9±0.3 cm fish-1 were raised for a 210-day experimental period using the following three treatments: (T1) seawater source in concert tanks, (T2) underground saltwater in concert tanks, and (T3) underground saltwater in cages (Wadi Maruit Lake). The results showed that fish grown in T1 exhibited better growth performance, FCR, immunity, kidney and liver enzyme activity, while no major differences regarding the histomorphology of the liver, gills and intestine between tested groups. Even though T1-reared fish recorded a better performance in terms of fish growth performance and feed utilization (FCR) compared to T2-reared fish, underground saltwater was proven to be suitable for marine aquaculture. This will open up new opportunities for marine fish farming in desert lands, as the variations in fish performance, FCR and health status between T1 and T2 are relatively small. Further research is needed on both the proper places that include brackish and/or full-strength saltwater that might be exploited in creating marine aquaculture projects, as well as the water quality's suitability for the target marine organisms, especially the content of heavy metals.

INTRODUCTION

Aquaculture is vital to meet the world's requirement for animal protein by conserving critically endangered fish such as sturgeons (**Vasilyeva *et al.*, 2019**) and producing around 50% of the world's seafood (**El-Kady *et al.*, 2022; Shahin *et al.*, 2022**). Furthermore, with over nine billion people expected to be on the earth by 2050, aquaculture would be required to grow five - fold to meet the increasing need for animal protein (**FAO, 2022**). Gross global aquaculture production was 122,600,000 tonnes (T) live weight in 2020, with a total value of US$281.5 billion (**FAO, 2022**). Egypt's

aquaculture was ranked the sixth in the world, with a total production volume of 1,591,900 T (**FAO, 2022; Abdel-Rahim *et al*., 2023**). Egypt's aquaculture is the greatest in Africa, accounting for 67.62% of the continent's overall aquaculture (**Abdel-Rahim *et al*., 2023**). Aquaculture production in Egypt accounts for approximately 80% of the total national fish production, with freshwater aquaculture accounting for more than 95% (**GAFRD, 2020**).

Currently, aquaculture is in danger of collapse due to regional changes surrounding Egypt and the hazards associated with fresh water scarcity, as well as the potential detrimental impacts of climate change (**GAFRD, 2020**). To maintain the ongoing growth and expansion of aquaculture, Egypt must discover alternative water sources to grow fish in new places, particularly euryhaline species since mariculture is a promising trend towards expanding fish production in Egypt despite concerns about freshwater scarcity. Egypt's marine aquaculture activities are focused in the northern governorates of Damietta, Port Said and Alexandria, as well as around the Suez Canal (**Shaalan *et al*., 2017**). The primary products of the Egyptian mariculture include flathead grey mullet (*Mugil cephalus*), gilthead sea bream (*Sparus aurata*), meagre (*Argyrosomus regius*) and the European seabass (**Shaalan *et al*., 2017; GAFRD, 2020; Elhetawy *et al*., 2021**).The geographical distribution of fish farms in Egypt suggests that the majority of them are located in northern governorates, the Delta zone, and around the Nile River's banks (**Shaalan *et al*., 2017; GAFRD, 2020**).

With Egypt already in a state of water scarcity, the government has inclined to exploit desert areas to maintain sustainable aquaculture development (**Farrag *et al*., 2021**). The majority of Egypt's geographical area is desert, with a significant reservoir of groundwater contributing 20% of the country's freshwater supplies; however, most aquifer systems contain large volumes of brackish groundwater **(Salim, 2012; Abd-Elaty *et al*., 2018**).This massive volume of groundwater helped create more than 120 industrial fish farms in desert regions (**Shaalan *et al*., 2017**). However, the Nile tilapia, along with the African catfish, carp and mullet, is estimated to account for 90% of desert aquaculture production (**Sadek, 2011; Shaalan *et al*., 2017; Adeleke *et al*., 2020**), while the aforementioned marine species including seabass are relatively absent from desert aquaculture.

Seabass is a large aquaculture facility in Europe, mainly in the Mediterranean countries, accounting for 96% of its gross production in 2016 (**Vandeputte *et al*., 2019**). The global seabass harvest has progressively increased from more than 60,000 T in 2003 to 243,900 T in 2020 (**FAO, 2022**). Egypt ranks the third among the largest producers, with 24,914 T, after Turkey and Greece, whose combined production accounts for over than 69% of global production, followed by Spain, Croatia and Italy, with 22,269 T, 6,220 T and 5,738 T, respectively (**FishstatJ, 2018**). Seabass is the third most important marine fish cultured in Egypt, after gilthead seabream (*Sparus aurata*) and meagre (*Argyrosomus regius*) (**GAFRD, 2020**). According to **GAFRD** (**2020**), the gross harvest

of seabass in Egypt was 34,477 metric T, with aquaculture accounting for almost 94% (32,555 metric T), lakes accounting for 5% (1664 metric T), and the Mediterranean Sea accounting for 1% (258 metric T) (**GAFRD, 2020**).

The seabass is euryhaline (0-40 ppt salinity) and eurythermal (2-32 °C) species and is often found in the coastal waters (100 m) from southern Norway (60°N) to the western desert (30°N), as well as in the Mediterranean and Black Seas (**Saillant *et al*., 2002**). Females have a fecundity of about 200.000 eggs/ kg and reproduce from December to March in the Mediterranean and from March to June in the Atlantic (**Vandeputte *et al*., 2019**). Since the mid-1980s, sea bass farming has continued after addressing early weaning survival (from 0 - 10% to 35 - 65%) with better running techniques, where it quickly displayed a high tolerance to intense rearing conditions (**Pérez-Ruzafa & Marcos, 2014**).

In Egypt, seabass farming techniques are comparable to those used for meagre (**Abdel- Rahim *et al*., 2019**) and gilthead seabream (**Lotfy *et al*., 2021**). Producers raise this species in earthen ponds, cages and a few farmers use concrete ponds (**Shaalan *et al*., 2017**). The farms where they are raised are private or linked to mega government projects such as the Canal Zone fish farming project and the Galion project near Kafr El-Sheikh (**GAIN Report, 2016; Shaalan *et al*., 2017)**. The water in which fish are raised has a significant impact on the biochemical, physiological and reproductive activities of the grown species. The purpose of this study was to compare the effects of utilizing two different groundwater sources in addition to seawater on the growth performance, health, and body composition of single-source seabass (generated by the Marine Fish Hatchery, K21, GAFRD).

MATERIALS AND METHODS

1. Study location, duration and fish samples

The present study was carried out in three private fish farms in Alexandria Governorate, Egypt, employing cages and cement tanks over a period of 210 days. In the current study, 27,000 apparently healthy *D. labrax* fingerlings, with an average initial weight of 12.0±0.25 g/fish and an average initial length of 10.9±0.3 cm/fish were used. In fish production units, juveniles were adapted for two weeks prior to starting the experiment. Each tank or cage was supplied by constant artificial aeration via a paddle wheel (1.5 kw).

2. The experimental design

This study aimed to evaluate the impact of three water sources in Egypt on the performance of *D. labra*, using a stocking density of 10 fish/m^3 in cages and tanks. Three treatments (T1, T2 and T3) were administered in triplicate as follows:

T1: Nine thousand seabass fingerlings were grown in three concrete tanks of 300m^3 water volume each (diameter 16 m x 1.5 m depth), using a seawater source with a salinity of 36-37 ppt.

T2: Nine thousand seabass fingerlings were grown in three cement tanks of 300m^3 water volume each (diameter 16 m x 1.5 m depth), using underground saltwater with a salinity of 36-38 ppt;
T3: Nine thousand seabass fingerlings were grown in three cages of 300 m^3 water volume each (diameter 10 x 20 x 1.5 m), using the underground saltwater of Wadi Mariut Lake, with a salinity of 10-18 ppt. Water flows continuously from the earth at T3, whereas the daily rate of water change was 25% at T1 and T2.

3. Feeding regime

The *D. labrax* juveniles were fed extruded diets with a 45/15% protein/fat ratio manufactured by ALLER AQUA FEED (https://www.aller aqua.com/) three times a day (8.30, 12.30, 16.30 hours.) in accordance with the recommended feeding level. For crude protein, crude fat, fiber, ash, NFE, and gross energy, the approximate analyses were 45, 15, 2.8, 7.2, 22%, and 20.9 MJ, respectively. Every two weeks, the daily feeding ratio was modified based on the body weights of the live fish. During the study months 1-2, 3-5, and 6-7, the pellet diameters were 1.5, 2 and 3 mm, respectively.

4. Water quality assessment

Table (1) shows the concentrations (µg/L) of some common heavy metals in the groundwater of the well (El-Max research station, NIOF), Lake Mariout water, and seawater (K21 region, Alexandria governorate). Water quality parameters were continuously monitored throughout the experiment period. Temperature, dissolved oxygen, pH, total ammonia nitrogen (TAN) and un-ionized ammonia were measured twice weekly for all treatments throughout the experiment. For water analysis, a SensoDirect 150 (Multiparameter, portable photometer) was used to measure ph/Redox, conductivity, dissolved oxygen, TDS and temperature (°C). The Hanna HI-97715 model was used to calculate total ammonia nitrogen (TAN) (portable photometer, Medium Range Ammonia, Hanna Instruments, Romania). Unionized ammonia was estimated using collected data for pH, temperature, salinity and TAN.

5. Growth performances, feed utilization and biometric indices

Fish were collected, counted and weighed at the end of the experiment. The following growth performance and feed utilization parameters were determined as follows:
Weight gain (g/fish), WG = Wt - W0, where: W0 = the initial mean weight of fish in grams; Wt = the final mean weight of fish in grams.; Average daily gain, ADG (gm⁄fish⁄day) = $Wt-W0$⁄days; Specific growth rate (%⁄day): SGR = $100\times\ln Wt - \ln W0$ ⁄days, where: ln: natural logarithm; Survival rate (%) = 100 * (Final number of fish ⁄ initial number of fish; Condition factor = 100 × (BW (g)/L^3 (cm)
Feed conversion ratio FCR- based on DM = feed intake (g) as dry weight DW) ⁄ weight gain (g).; Protein efficiency ratio PER = weight gain (g) ⁄protein intake (g).; Protein

productive value PPV% =100 × (protein gain (g)/protein intake (g)).; Energy gain Kcal = Et −E0, where: *E*t: Energy content in the fish carcass (Kcal) at the end, *E*0: Energy content in the fish carcass (Kcal) at the start; Energy utilization EU% = 100 × (Energy gain (kcal)/Energy intake (kcal)).

Carcass energy kcal/100g DM = protein%×5.64 +Ether extract%×9.44. (AOAC, 2000)

By the end of the experiment, four fish from each treatment were sacrificed to get their final biological records, including liver and viscera weights to calculate hepatosomatic (HSI) and viscerosomatic (VSI) indices as follows:

Hepatosomatic index, HSI = 100 * [liver weight (g)/total body weight (g)] according to Schreck & Moyle (1990);

Viscerosomatic index, (VSI) = 100 * [total weigh of all viscera (g)/total body weight of the fish before removal of the viscera (g)].

Table 1. Some concentrations (µg/L) of common heavy metals in the well groundwater (El-Max research station, NIOF), Lake Mariout water, and seawater (K21 region, Alexandria governorate)

Heavy metal	Seawater	Well water	Mariout lake water
Fe	35 ± 1.00	99.3±2.2	682.70 ± 248
Mn	4 ± 0.001	85.2±1.08	58.91 ± 22
Zn	4 ± 0.001	6.5±0.002	60.42 ± 31
Cu	4 ± 0.001	5.3±0.005	12.40 ± 4
Ni	7 ± 0.001	70.0±5.0	2.09 ± 3
Pb	6 ± 0.003	28.0±3.0	33.55 ± 11
Cd	6 ± 0.005	40.0±1.0	6.35 ± 1

Data source: **(El-Dahhar et al., 2021; El-Degwy et al., 2023)**.

6. Blood hematological and biochemical analysis

At the end of the experiment, blood samples were taken from the caudal vertebral vein of an anesthetized fish (three fish from each group in triplicate). The blood was divided into two tubes, one of which included EDTA as an anticoagulant for hematological examination. The other was separated by centrifuging coagulated blood at 4000 rpm for 15 minutes at 4°C without anticoagulant and stored at 20°C until analysis. The erythrocytes, leukocytes and hemoglobin were counted according to the technique outlined by **Stoskopf** (**1993**). The concentration of hemoglobin was measured using the cyanomet hemoglobin technique using Drabkin's solution according to **Stoskopf** (**1993**). Using ferricyanide and cyanide ion, the cyanomet hemoglobin technique transforms all hemoglobin derivatives to methemoglobin. Methemoglobin is a stable red substance that may be quantified by colorimetry. According to **Dacie and Lewis** (**1991**), the

microhematocrit technique was utilized to calculate the PCV percentage. Thin blood films were made, air dried, fixed with methanol for 3-5 minutes, stained with Gimsa stain for 8-10 minutes and then allowed to dry to calculate differential leukocytic count (DLC). According to a hundred blood smears, the white blood cell count was determined (**Stoskopf, 1993**). The absolute DLC was determined using the following formula according to **Thrall *et al.*** (**2004**): Absolute DLC = number of each white cell multiplied by the total leukocyte count per 100.

Serum glucose was assessed using the methods described by **Foster and Dunn** (**1974**). Cholesterol was detected using the method of **Allain *et al*.** (**1974**). Total albumins and proteins and albumins were determined according to **Doumas *et al.*** (**1972**) and **Doumas *et al.*** (**1981**), respectively. Serum globulin was determined by subtracting the albumin value from the total protein value of the same sample. Serum aspartate aminotransferase activity (AST) and alanine aminotransferase activity (ALT) were estimated according to the method described by **Reitman and Frankel** (**1957**). Alkaline phosphatase (ALP) activity was determined using an enzymatic colorimetric method according to **Tietz *et al.*** (**1983**). Uric acid was estimated according to the method described by **Whitehead *et al.*** (**1991**). Serum creatinine was determined using the Colori-metric method (**Heinegård & Tiderström, 1973**).

7. Histology

The gills, liver and intestine (middle section) of fish from different groups were collected and then fixed in 10% neutral buffered formalin. The tissues were embedded in paraffin and sectioned to a thickness of 5µm following dehydration and cleaning. The serial sections were stained with hematoxylin and eosin (**Bancroft *et al.*, 2013**). Histomorphometric analysis was performed using ImageJ analysis software (National Institutes of Health, MD, USA).

8. Statistical analysis

Using the software programme, the data were statistically examined (SPSS version 26). A one-way analysis of variance (ANOVA) was used to assess the effect of different water sources on growth performance, feed utilization, chemical composition, blood biochemical assays, immunity and histology of sea bass fingerlings. Differences within each treatment were examined using the Duncan test with a probability of 0.05.

RESULTS

1. Water quality criteria

As shown in Table (2), the continuous monitoring of water quality parameters revealed significant differences ($P<0.05$) regarding salinity, dissolved oxygen, NO_3, total nitrogen (NH_3 - N), PO_4, total phosphorus and Chlorophyll "a" between the different treatments. There were no significant differences ($P>0.05$) between T1 and T2 regarding the measured parameters of salinity, Do, PO_4, total phosphorus and Chlorophyll "a".

Table 2. Water quality parameters in *D. labrax* growing units with different water sources during the 210- day trial

Parameter	Treatments		
	T1	T2	T3
Temperature (0C)	24.15±0.89[a]	24.35±1.24[a]	22.65±3.55[b]
DO_2	6.45±0.26[b]	6.35±0.21[b]	7.95±1.88[a]
pH	8.11±0.02	8.01±0.06	8.03±0.16
Salinity (ppt)	36.2±0.17[a]	37.6±0.26[a]	14.0±4.31[b]
NH_3 (mg/l)	0.055±0.01[b]	0.080±0.02[a]	0.050±0.02[b]
NO_2 (mg/l)	0.050±0.01	0.070±0.01	0.065±0.03
NO_3 (mg/l)	0.305±0.04 [c]	0.570±0.11[b]	1.095±0.26[a]
Total nitrogen (mg/l)	0.505±0.07[c]	0.820±0.17[b]	1.300±0.31[a]
PO_4 (mg/l)	0.090±0.02[c]	0.165±0.04[b]	0.305±0.07[a]
Total phosphorus (mg/l)	0.19±0.02[c]	0.51±0.06[b]	1.36±0.35[a]
Chlorophyll "a" (µg/l)	4.5±2.02[c]	19.5±4.33[b]	101.0±31.18[a]

Data are presented as mean ± S.D. Values within a row with different superscripts differ significantly ($P< 0.05$). T1: *D. labrax* reared in tanks using seawater source; T2: *D. labrax* reared in tanks using ground saltwater; T3: *D. labrax* reared in cages using ground saltwater of Wadi Maruit Lake.

2. Growth indicators and feed utilizations

Table (3) depicts the growth performance and feed utilization of *D. labrax* raised under three water sources for 7 months. The presented data demonstrate the significant superiority of the growth parameters for *D. labrax* juveniles in T1, with the highest values of final weight (FW), weight gain, ADG, SGR and survival rate. It was noted that, the highest values of growth indicators were recorded for *D. labrax* in T1, followed by T2, while the lowest values were recorded for T3. Concerning feed utilization, the best FCR and PER were recorded for fish raised at T1, while the worst were reported for fish grown in T3. Moreover, PPV % was increased significantly ($P<0.05$) for T1, while no significance was recorded between T2 and T3. Energy utilization % recorded significant values for T1, while the energy gain was significant with T2 and T3.

Table 3. Growth performance, survival rate and feed utilization of *D. labrax* reared under different water sources for 210 days

Treatment/parameters		T1	T2	T3
Growth performance	FW, gm/fish	114.85±1.19^{a}	106.38±1.54^{b}	75.99±2.14^{c}
	Gain, gm/fish	102.85±1.35^{a}	94.25±1.44^{b}	63.99±1.93^{c}
	ADG, gm/fish/day	0.49±0.01^{a}	0.45±0.01^{b}	0.30±0.01^{c}
	SGR, %/fish/day	1.08±0.011^{a}	1.03±0.01^{b}	0.88±0.01^{c}
	Survival, %	94.89±0.3^{a}	90.3±0.5^{b}	88.2±0.2^{c}
	Condition factor	1.16±0.02	1.12±0.04	1.06±0.03
Feed utilization	FCR, gm	1.54±0.02^{c}	1.65±0.01^{b}	1.76±0.02^{a}
	PER, gm	1.47±0.03^{a}	1.37±0.0^{b}	1.28±0.01^{c}
	PPV, %	27.82±0.35^{a}	22.31±0.20^{b}	21.66±0.15^{b}
	Energy Gain, kcal/100gm	110.03±3.50^{b}	164.44±2.93^{a}	155.02±1.30^{a}
	Energy Utilization, %	23.04±0.12^{a}	19.87±0.14^{b}	19.22±0.06^{c}

Data are presented as mean ± S.D. Values within a row with different superscripts differ significantly ($P< 0.05$). T1: *D. labrax* reared in tanks under seawater source; T2: *D. labrax* reared in underground saltwater tanks; T3: *D. labrax* reared in cages using a saltwater (underground) of Wadi Maruit Lake.

3. Morphological parameters of the digestive system

As shown in Table (4), the water source significantly affected the morphological digestive system of *D. Labrax*. The data given for the measured parameters show significant values of VSI, HIS, intestinal length / total length, and intestinal length / intestinal length in favor of T1. Regarding T2 and T3, HIS and VSI increased significantly for T2, while intestine length/total length and gut length/intestine length were in favor of T3.

Table 4. Morphological digestive system of European sea bass, *D. labrax* reared under different water sources for 210 days

Treatment/parameters		T1	T2	T3
Morphological digestive system	HIS, %	1.52±0.10[a]	1.43±0.15[b]	1.30±0.20[c]
	VSI, %	9.13±1.13[a]	8.41±0.81[b]	7.87±0.79[c]
	Intestine length/total length, %	97.55±11.57[a]	85.40±12.94[c]	86.58±20.85[b]
	Gut length/intestine length, %	18.56±1.19[a]	13.26±0.85[c]	17.76±2.81[b]

Data are presented as mean ± S.D. Values within a row with different superscripts differ significantly ($P< 0.05$). T1: *D. labrax* reared in tanks under seawater source; T2: *D. labrax* reared in underground saltwater tanks; T3: *D. labrax* reared in cages using a saltwater (underground) of Wadi Maruit Lake.

4. Hematology and serum biochemistry

4.1. Blood and serum analysis

For seabass hematology, it was significantly affected ($P\leq 0.05$) by the water source as shown in Table (5). Hemoglobin, RBCs, WBCs, Neutrophils, Monocyte, hematocrit, cholesterol and triglyceride levels were significantly ($P\leq 0.05$) increased for T1, compared to T2 and T3. While, MCV (µm³/cell) and lymphocytes increased significantly in T2. Remarkably, no significant difference was detected between T2 and T3 regarding hemoglobin.

Table 5. Changes in blood and serum analyses in *D. labrax* reared under different water sources for 210 days

Blood parameter	T1	T2	T3
Hemoglobin (Hb)	10.88±0.62[a]	9.15±0.47[b]	9.23±0.58[b]
Red blood cells (RBCs) ($x10^6$ µ/L)	6.32±0.45[a]	4.62±0.39[b]	3.22±0.34[c]
White blood cells (WBCs) ($x10^3$ µ/L)	33.18±4.82[a]	31.42±4.13[b]	27.46±3.34[c]
Neutrophils (%)	11.0±0.6[a]	10.0±0.6[c]	10.5±0.3[b]
Lymphocyte (%)	80.5±0.3[b]	83.0±0.6[a]	79.0±0.6[c]
Monocyte (%)	7.0±0.00[a]	5.9±0.29[b]	4.5±0.29[c]
Hematocrit ((hct)) (%)	37.17±2.54[a]	34.13±3.11[b]	32.16±2.85[c]
MCV (µm³/cell)	122.0±0.0[b]	123.1±0.1[a]	121.9±0.1[b]
Cholesterol (mg/dl)	291.65±1.33[a]	283.2±1.07[b]	270.12±1.25[c]
Triglyceride (mg dL^{-1})	304±2.66[a]	295±2.14[b]	290±1.57[c]

Data are presented as mean ± S.D. Values within a row with different superscripts differ significantly ($P< 0.05$). T1: *D. labrax* reared in tanks under seawater source; T2: *D. labrax* reared in underground saltwater tanks; T3: *D. labrax* reared in cages using a saltwater (underground) of Wadi Maruit Lake.

4.2. Immune parameters

The immunity of seabass grown in various water sources showed considerable changes between treatments, as presented in Table (6). Fish reared in T1 and T2 exhibited noticed levels of serum immune parameters as total protein and alkaline phosphate increased significantly for fish in T1, and glucose increased significantly for fish in T2, while no significant differences were recorded between T1 and T2 with regard to globulins. The lowest values of serum immune parameters were reported for fish raised in T3.

Table 6. Immune parameters of European sea bass reared under different water sources for 210 days

Serum immune parameter	Treatments		
	T1	T2	T3
Total protein (mg dL-1)	7.12 ± 1.12^{a}	6.44 ± 0.85^{b}	5.66 ± 0.31^{c}
Glucose (mg dL^{-1})	75 ± 3.45^{b}	104 ± 5.37^{a}	50 ± 3.85^{c}
Globulin	$3.19\pm0.02^{\mathbf{a}}$	$3.08\pm0.03^{\mathbf{a}}$	$2.56\pm0.65^{\mathbf{b}}$
Alkaline phosphate	$55.5\pm1.5^{\mathbf{a}}$	$40.5\pm2.5^{\mathbf{b}}$	$40.5\pm0.5^{\mathbf{b}}$

The values with a different superscript in the same row are significantly different ($P\leq0.05$).

4.3. Kidney serum enzymes

Seabass kidney function indicators were significantly affected by the applied water source, as shown in Table (7). In general, the lowest activity of kidney serum enzymes was reported for fish grown in T1, compared to T2 and T3. The highest activities of kidney function indicators of urea and creatinine were noticed in T3 and ammonia in T2, while T2 and T3 shared the same significant level of uric acid.

Table 7. Kidney enzymes of European sea bass reared under different water sources for 210 days

Serum kidney parameter	Treatments		
	T1	T2	T3
Urea (mmol/L)	$18.5\pm0.87^{\mathbf{c}}$	$23.9\pm0.87^{\mathbf{ab}}$	$25.2\pm0.86^{\mathbf{a}}$
Creatinine (mg/dl)	0.59 ± 0.01^{c}	0.65 ± 0.08^{b}	0.72 ± 0.01^{a}
Uric acid (mg/dL)	$1.18\pm0.03^{\mathbf{b}}$	$1.68\pm0.03^{\mathbf{a}}$	$1.68\pm0.07^{\mathbf{a}}$
Ammonia (mmol/L)	$59.4\pm3.46^{\mathbf{b}}$	$60.0\pm1.15^{\mathbf{a}}$	58.42 ± 0.58^{c}

The values with a different superscript in the same row are significantly different ($P\leq0.05$).

4.4. Liver serum enzymes

Liver serum enzymes (AST, ALT, ALP) of seabass grown under different water sources varied greatly, as presented in Table (8). Overall, less liver enzyme activity was observed with fish raised at T1, compared to fish in T2 and T3. The highest levels of all liver enzymes were reported for fish reared in T3, followed by T2.

Table 8. Liver enzymes (AST, ALT, ALP) of European sea bass reared under different water sources for 210 days

Liver enzyme	Treatments		
	T1	T2	T3
AST(U/L)	81.34±0.24^{c}	83.12±0.15^{b}	85.06±0.41^{a}
ALT(U/L)	50.13±0.55^{c}	52.65±0.39^{b}	55.01±0.45^{a}
ALP(U/L)	1.25±0.08$^{\mathbf{c}}$	1.61±0.04$^{\mathbf{b}}$	2.15±0.06$^{\mathbf{a}}$

The values with a different superscript in the same column are significantly different ($P \leq 0.05$).

5. The whole-body chemical composition

The results of *D. labrax* carcass analysis are presented in Table (9). Statistical analysis revealed that the water source used to rear the seabass fingerlings had no significant impact on the carcass composition. The major components of carcass protein, lipid and dry matter did not change significantly between seabass in different water sources. Carcass ash differed significantly for fish reared in T3, while carcass energy varied significantly for fish reared in T2.

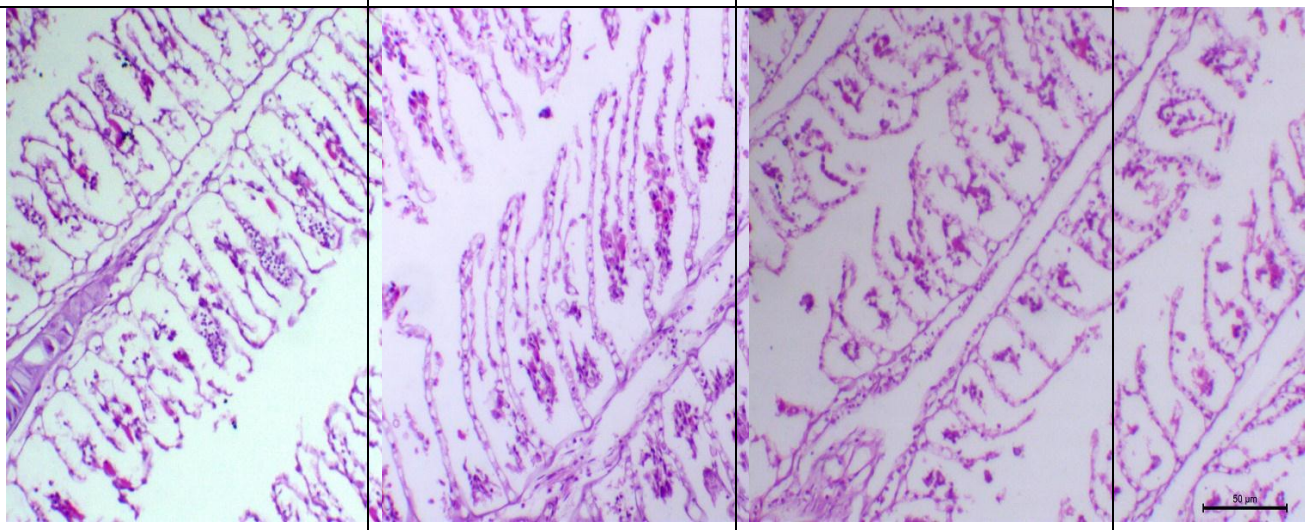
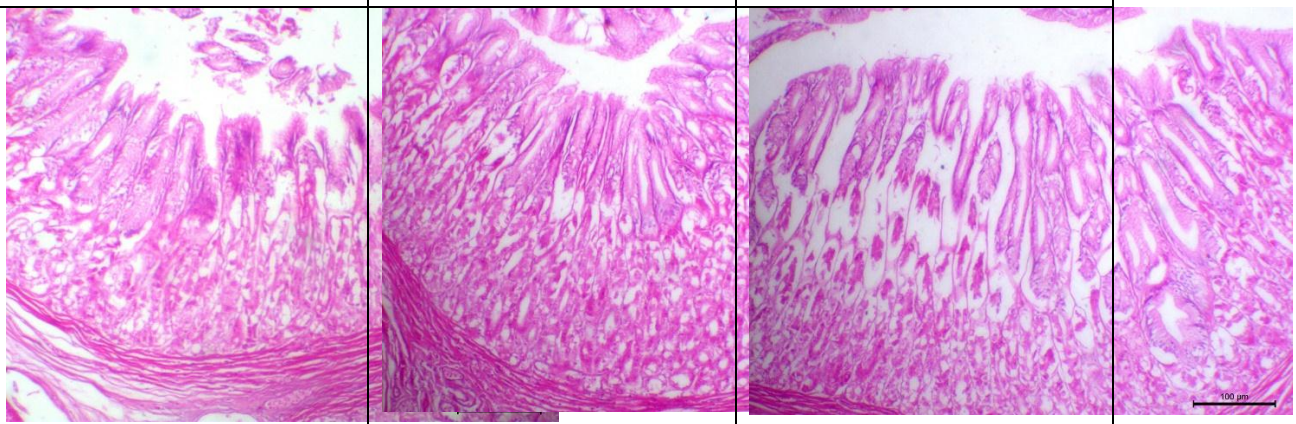
Table 9. Carcass composition of *D. labrax* L reared under different water sources for 210 days

Carcass composition	Initial	Final		
		T1	T2	T3
Dry matter, %	32.78±0.17	33.60±0.25	33.20±0.21	33.35±0.22
Protein, %	56.33±0.35	53.8±0.11	53.70±0.27	53.69±0.22
Ether extract, %	25.90±0.18	28.15±0.09	28.17±0.15	28.77±0.20
Ash, %	15.93±0.22	17.19±0.39$^{\mathbf{c}}$	17.41±0.19^{b}	17.92±030$^{\mathbf{a}}$
Carcass-energy, Kcal/100gm	-	567.1±0.75^{a}	566.9±0.71^{a}	544.4±2.63^{b}

Values are expressed as the mean ± SE. Different letters in the same row indicate significant differences among the treatments ($P \leq 0.05$).

6. Histomorphology

Fig (1) shows the histological findings of the gills, middle intestines and liver of seabass fingerlings raised under different water sources. With respect to the results of the microscopic examination, the different water sources used for rearing seabass juveniles did not significantly affect the internal organs of fish. Fish gills showed normal secondary gill lamellae in the three groups. The intestinal villi (middle section) exhibited long and branched villi lined with a pseudo-stratified epithelium with goblet cells for fish in T1, and a marked increase in intestinal villi length with increased goblet cells within the lining epithelium for fish in T2 in addition to an increase in intestinal villi length with fish from T3. The fish liver from all groups showed a normal histomorphology with hepatic vacuolation consistent with fatty feed and a minimal decrease of hepatic vacuolation in favor of T3, while all groups showed normal pancreatic portions.

Organ	**T1** (Seawater)	**T2** (Well groundwater)	**T3** (Lake groundwater)
Gills			
Intestine (middle)			

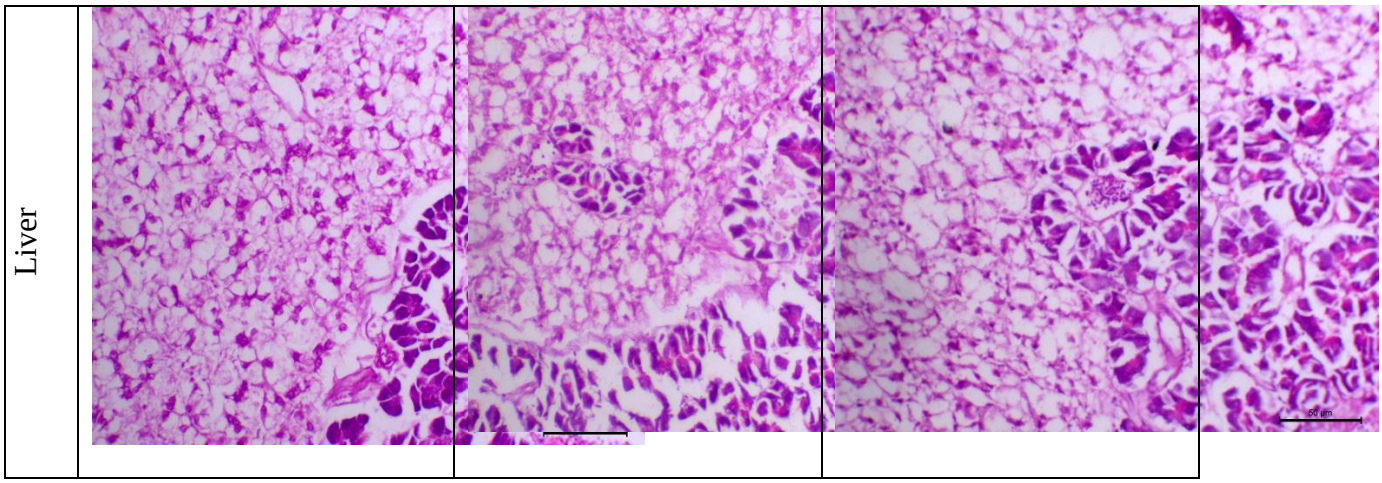


Fig. 1. Histological characteristics of the gills, intestine (middle) and liver of seabass raised under different water sources for a 210-day experimental period

Gills of fish in the three groups showed normal secondary gill lamellae with mild basal adhesion, H&E, X200, bar= 50 µm.

Intestine (middle section) exhibited long and branched villi lined with pseudostratified epithelium with goblet cells for fish in T1, a marked increase of intestinal villi length with increased goblet cells within the lining epithelium for fish in T2, and increase in the length of intestinal villi with fish from T3.

Liver in all groups showed a normal histomorphology with hepatic vacuolation consistent with fatty feed with a minimal decrease of hepatic vacuolation in favor of T3, while all groups showed normal pancreatic portions.

DISCUSSION

The health and growth of fish are directly related to the quality of the water in which they are raised as well as the culture system used (**Viadero, 2005; Elhetawy *et al*., 2020**). Physical, chemical/biochemical, or a mix of these factors influence fish growth and productivity. Although each of the water parameters has an individual impact, the cumulative effect of these factors on fish growth and production is interrelated (**Viadero, 2005**). Although significant differences ($P<0.05$) were observed in this study, all water variables fluctuated within the acceptable levels for euryhaline species (**Killen *et al*., 2007**; **Chang *et al*., 2021**). The three water sources used in this trial provided a suitable environment for raising seabass. Excluding the influence of some water quality parameters (temperature C^0, DO_2, pH, NH_3, NO2, and NO_3) and focusing on the content of heavy metals in the water (Table 1), salinity, total phosphorous content and PO4 are indicators of the presence of contaminants in the water. Changes in *D. labrax* growth and survival rates could be explained by the fact that water salinity and contaminant concentration had a significant effect on fish performance in the three groups. The lower survival rate and the decreased final weight reported in T3 are associated with lower salinity and the presence of contaminants. Water in T3 had higher concentrations of total

phosphorus (1.36 mg/l), PO4 (0.305 mg/l) and total nitrogen (1.300 mg/l) than T1 and T2. In addition, substantial concentrations of sulfate (11.4-20.6 ppm) and mercury (0.08-0.4 ppm) were detected in Wadi Maruit Lake (**El-Ebiary *et al.*, 2015**). The adverse effects of these high levels of heavy metals and pollutants undoubtedly impeded the growth and survival of seabass.

Regarding the survival and growth performances of seabass, the first aspect of the current experiment is that the various water sources performed well in terms of survival and growth, which were significantly affected, with T3 recording the lowest values and T1 the best. This growth and survival result is in exact agreement with the findings of **Lotfy *et al.*** (**2021**) for juvenile *S. aurata* cultured for eight months under similar conditions (seawater, well groundwater and Wadi Maruit lake groundwater).

In the same context, these results are supported by the findings of **González-Félixa *et al.*** (**2017**) for totoaba (*Totoaba macdonaldi*) and Gulf corvina (*Cynoscion parvipinnis*) grown under salinity levels ranging from 2 to 35, with the lowest survival rates recorded at salinities below 12.3 and 13.3, respectively. However, our findings contradicts to some extent those reported for *S. aurata* larvae kept at low salinity (20‰) for two months; however, they are essentially identical in terms of length, weight and survival rate to those grown at sea salinity (**Azab *et al.*, 2005**). On the contrary, **Klaoudatos and Conides** (**1996**) assessed that brackish water is ideal for rearing euryhaline fish such as *D. labrax* and brown spotted grouper (*Epinephelus chlorostigma*), and added that the best survival rate for *S. aurata* was recorded with a low salinity of 8 compared to saltwater.

For seabass growth and feed utilization, the data reported here illustrate the significant impact of water quality at each source, with T1 having superior final weight, ADG, SGR and FCR, compared to T2 and T3. The optimal salinity level for maximum growth of seabass juveniles is 34-35 ppt (**Person-Le Ruyet *et al.*, 2004**), and these results are identical to those reported for *S. aurata* fingerlings (11 g) raised for 240 days in the same setting (**Lotfy *et al.*, 2021**). Similarly, the highest final weight (28.07g) and lowest FCR (31.49%) were recorded for meagre juveniles at salinities greater than 20 (**Abdel- Rahim *et al.*, 2019**).

In a different context, **Nathanailides *et al.*** (**2010**) found that the growth rate of juvenile *D. labrax* (7 g) was similar at ambient temperatures in freshwater tanks and marine cages, and **Yilmaz *et al.*** (**2020**) found that *D. labrax* raised for 60 days in freshwater at 25°C had the highest FW (83.8 g) and SGR (1.6% day-1), compared to 20 ppt and 38 ppt. Although T1 had the highest FCR value (1.54), which was significantly higher than T2 and T3, the values for the three groups varied between the ranges reported for seabass (1.4-1.6) (**Andrew *et al.*, 2005**). Similar to the findings of **Lotfy *et al.*** (**2021**) for *S. aurata* fingerlings, the best energy gain, PER and PPV were recorded for T1, whereas the worst values were obtained for T3. In a different context, seabass events (31.4g) raised for 2 months in freshwater had a mean FCR of 1.2 and PER of 1.6

compared to FCR of 1.4 and PER of 1.6 for those raised at 38 salinity (**Yilmaz *et al.*, 2020**), while *D. labrax* juveniles (1.6g) grown for 600 days at 7 salinity had a mean FCR of 1.72 and PER of 1.24 (**Altan, 2020**).

The differences in nature between groundwater and seawater in terms of physical and chemical properties, as well as in the balance of components can be used to explain changes in growth and FCR between the three groups. This difference also increases the energy lost in various physiological processes. Furthermore, the presence of pollutants and the decrease in salinity below optimal levels in T3 (recorded less than 10 ppt) had a negative impact on feed intake, resulting in lower FCR and SGR. Fish that are adapted to low-salinity water lose their ions, and this loss must be compensated by an uptake from the water or the feed. Maintenance requirements have been shown to increase as salinity decreases (**Conides & Glamuzina, 2006**). Therefore, the euryhaline species demonstrated good growth rates when reared in decreasing salinity and fed diets containing salts that met the osmotic needs and contributed to providing the energy needed for osmoregulation (**Azab *et al.*, 2005**). According to the deviation of salinity from the isosmotic dot, the energy used in osmosis regulation has a salinity effect on the growth of numerous euryhaline species that ranges from less than 10% to 50% (**Furspan, 1981; Morgan & Iwama, 1999**). As a result, this energy cannot be used for growth (**Wootton, 2012**). Furthermore, the total energy expended by osmoregulatory has been estimated to range between 20% and 68% in different species, but these values should include not only the actual cost of ion transport but also the energy used by other metabolic processes that respond to fluctuations in salinity **(Ordóñez-Grande *et al.*, 2021**).

The morphology of fish digestive systems reflects the influence of their diets and the environments in which they live. In this case, because *D. labrax* was fed the same diet, changes in the morphology of the digestive system could be attributed to the effects of the ambient water in which they grew. The T1 fish had significantly higher hepatosomatic (HSI) and viscerosomatic (VSI) indices than the T2 fish. This clearly indicates the effect of water supply and its phytoplankton, zooplankton, and pollution content on the development of the digestive tract in carnivorous fish. This finding contrasts with the findings of **Lotfy *et al.*** (**2021**) for *S. aurata*, who found a significant increase in VSI with fish in groundwater and an increase in HSI with fish in seawater. The authors attributed these findings to the environment and culture system used with *S. aurata*. The higher values achieved in this trial with seawater and well water could be attributed to an increase in liver metabolic activity. **Sadekarpawar and Parikh** (**2013**) explained these results as a result of the high binding properties that boost digesting viscosity and, as a result, metabolic rate.

Hematology in fish is critical in aquaculture research because it draws attention to the health and welfare of cultivated organisms. Hematological parameters are used as indicators of the influence of environmental factors on physiological homeostasis, such as

the effects of salinity, temperature, food abundance, seasonal pattern, age and sex of the fish, and any change in blood criteria is considered a preliminary response to stress (**Abdel- Rahim *et al.*, 2019; Murmu *et al.*, 2020**). Numerous studies have been conducted to determine the effects of salinity on fish development, physiology, reproduction and metabolism. Changes in water salinity cause morphological, biochemical and endocrinological variations in fish (**Jahan *et al.*, 2019; Hossain *et al.*, 2022**). These variations result in changes in oxygen consumption and energy requirements (**Semra *et al.*, 2013**).

In this study, the recorded values of hemoglobin (Hb), red blood cells (RBC), white blood cells (WBCs), hematocrit (HCT), cholesterol and triglyceride demonstrated that fish raised in T3 had lower levels of these hematological and biochemical parameters, compared to fish raised in T1 and T2. This observation can be due to the reduced salinity caused by ion leakage from the plasma during the fish's energy metabolism (**Soltanian *et a*l., 2016; Elarabany *et al.*, 2017**). These findings are consistent with those of **Soltanian *et al.*** (**2016**), who discovered a substantial effect of salt levels on RBCs, HCT and Hb in fish. **Goda *et al.*** (**2019**) found that low salinity reduces (RBC, WBC, Hb, HCT, and TP) in seabass. While, **AbdelRahim *et al.*** (**2019**)**,** found that increasing water salinity from 8 ppt to 32 ppt increased hemoglobin, cholesterol and triglyceride levels in meagre fish. These findings contradict the report of **Lotfy *et al.*** (**2021**) on *S. aurata*. In terms of immunohistochemistry for TP, glucose, globulins and alkaline phosphate, the fish grown in T2 had higher levels of glucose and globulin, while the fish grown in T1 had higher levels of TP and alkaline phosphate, indicating that differences in water criteria and salinity did not affect fish immunity. On the other hand, serum TP is an important clinical indicator of the fish's nutritional state, health, stress level and liver function. Serum TP also contains nonspecific immunological components such as immunoglobulins (**Magnadóttir, 2006; Abdel- Rahim *et al.*, 2019**). In this study, TP levels in T1 and T2 increased as salinity increased; hence, high salinity resulted in enhanced immune responses in sea bass. The fundamental source of energy for all metabolic processes including osmoregulation is glucose produced by carbohydrate metabolism (**Perry & Walsh, 1989; Morgan *et al.*, 1997**). Glucose is the carbohydrate source that is stored for all metabolic activity. In the current study, the glucose concentration in groundwater decreased with decreasing salinity. This could occur because osmoregulation requires more energy at lower salinity levels. These findings are consistent with the findings of **Xavier *et al.*** (**2021**). The effect of increased TP in this study seems to be the same as that documented for *S. aurata* **(Laiz- Carrión *et al.*, 2005; Lotfy *et al.*, 2021**) and meagre (**Abdel- Rahim *et al.*, 2019**).

Kidney function indicators (urea, uric acid, creatinine and ammonia) and liver enzymes (AST, ALT, and ALP) are significant diagnostic biomarkers frequently used to monitor fish health and condition (**Azodi *et al.*, 2021**). In the current study, fish raised in T1 had lower plasma liver and kidney enzyme activities than fish raised in T2 and T3,

while the levels of these enzymes in all treatments were normal and are consistent with previous studies that found that fish in good condition had low levels of the prior enzymes, and vice versa (**El-Kady *et al.*, 2022**). Increased AST and ALT levels in the blood may be caused by a damaged liver though kidney and gill damage may also be present **(Shahsavani *et al.*, 2010)**. The levels of previous enzymes herein agree with those published for *S. aurata* (**Lotfy *et al.*, 2021**). However, they disagree with those reported for meagre fish (**Abdel- Rahim *et al.*, 2019**). The higher levels of these enzymes in T3 compared to T1 and T2 may be due to the reduced salinity of the water and the presence of pollutants, which resulted in an increased metabolic rate during osmoregulation and stress (**Goda *et al.*, 2019**).

The whole-body chemical composition of fish has been observed to change with changes in salinity, temperature, fish size, diet type, diet quality and developmental stage (**Castillo-Vargasmachuca *et al.*, 2017**). Chemical analysis of seabass carcass demonstrated that the change in water sources had no significant effect on the basic components of protein, fat and dry matter. The current study's findings are consistent with those of **Sardella and Brauner** (**2008**) and **Perez-Velazquez *et al.*** (**2014**), demonstrating that seabass is a good osmoregulator. Nevertheless, these findings conflict with those reported for the spotted rose snapper *Lutjanus guttatus* (**Castillo-Vargasmachuca *et al.*, 2017**). Furthermore, the crude protein and fat contents of meagre fish were found to be sensitive to the salinity of the farming water (**Abdel- Rahim *et al.*, 2019**). Furthermore, **Kumar *et al.*** (**2016**) discovered that the body composition of *Pangasianodon hypophthalmus* changes when salinity increases within saline water. Differences in the effects of salinity on the chemical composition of the seabass body and other species could be explained by the fact that the seabass is a better osmoregulator than other marine fish and can tolerate a wide range of salinity and temperature, making it less susceptible to stress and thus requiring less energy for metabolism. Furthermore, in marine species such as *Cynoscion parvipinnis* and *Totoaba macdonaldi*, crude protein and fat levels do not appear to be sensitive indicators of osmotic stress (**González- Félix *et al.*, 2017**).

The healthy development of the internal organs of fish, particularly the intestines and liver, has a significant impact on growth, disease resistance and feed efficacy (**El-Kady *et al.*, 2022**). Microscopic examination revealed normal and healthy morphology of the middle intestine, liver, and gills of *D. labrax* in all groups, with no significant differences seen in the organs investigated related to the different water sources. This conclusion can be linked to the ability of seabass to resist salinity change as a result of their yearly life cycle movement from seawater to brackish and even freshwater (**Kokou *et al.*, 2019; Vandeputte *et al.*, 2019**). These findings are congruent with those of Azodi ***et al.*** (**2021**) but contradict those of **Tran-Ngoc *et al.*** (**2017**), who found variations in gut structure in fish exposed to low versus high salinity. Furthermore, environmental stressors such as salinity and heavy metal load can cause changes in intestinal

morphology (**Tran-Ngoc *et al.*, 2016**). Due to its functional position, the fish liver is a fundamental digestive organ connected with a reliable biomarker showing the health status of fish (**Sharmin *et al.*, 2021**). It is primarily associated with detoxification and biotransformation and regulates blood circulation (**Van der Oost *et al.*, 2003**). The fish in this study displayed normal liver function in all three water sources used, which could be explained by the fact that the seabass is a euryhaline and eurythermal species; moreover, the water in which they were grown was appropriate, and the fish were not exposed to harmful pathogens in any of the three sources.

CONCLUSION

According to the findings of the current study, the underground saltwater is an ideal source for establishing a mariculture enterprise in desert environments. Fish raised in tanks using full-strength underground saltwater had higher FW, SGR, FCR, immunity and healthier inner organs than those raised in lake underground brackish water, and they were comparable to fish raised in seawater. To begin mariculture in the desert, more research on the water quality of underground saltwater is recommended to evaluate the water quality parameters in various areas that could represent potential sites for aquaculture. Based on the findings of this study, it is possible to conclude that saline groundwater or brackish groundwater is an appropriate supply for growing seabass in Egypt's desert and anywhere in the world. However, further research is needed on both proper places that include brackish and/or full-strength saltwater that might be exploited in creating marine aquaculture projects, as well as the water quality's suitability for the target marine organisms, especially the content of heavy metals.

Animal Care Protocol: The research experiment was approved by NIOF Committee for Institutional Care of Aquatic Organisms and Experimental Animals (NIOF-IACUC), approval N^0 NIOF-AQ1-F-23-R-002, Egypt.

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data curation and supervision. **Mohamed Abdel-Rahim**, the experimental idea, formal analysis, validation, visualization, conceptualization, manuscript revision.

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