EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
PHYSIOLOGY & MOLECULARBIOLOGY

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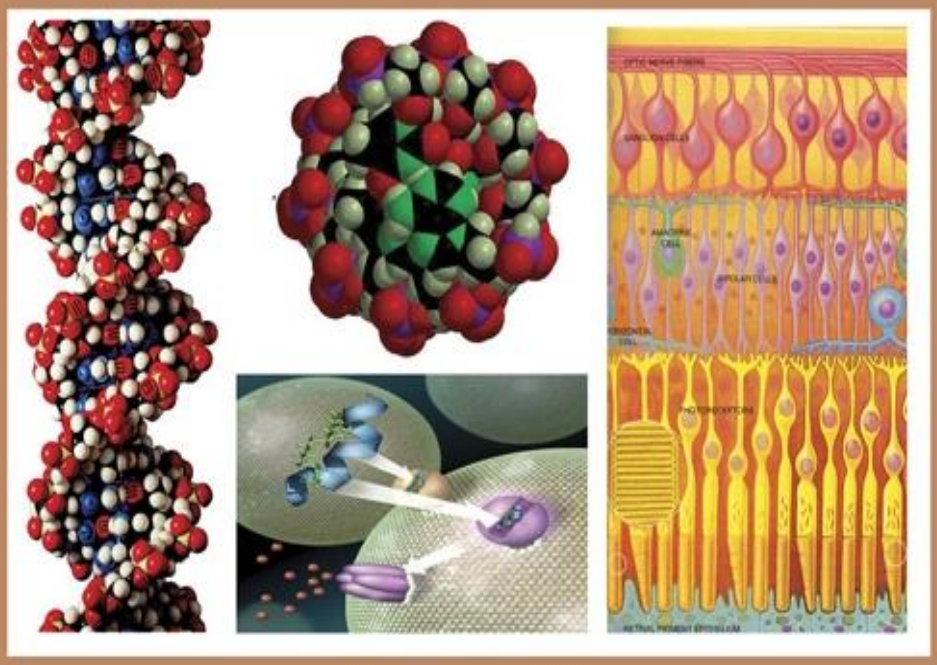

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 15 No. 1 (2023)

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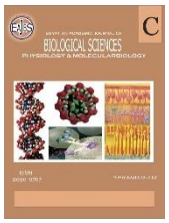
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp21-26 (2023)*
DOI: 10.21608/EAJBSC.2023.280112

Egypt. Acad. J. Biolog. Sci., 15(1):21-26 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Morphology and Molecular Identification of Microalgae *Dunaliella salina* Strain Seq Duna5.8S Isolated from an Algerian Salt Lake

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ARTICLE INFO

Article History
Received:28/12/2022
Accepted:13/1/2023
Available:17/1/2023

Keywords:
Dunaliella salina, Algeria, morphology, ARNr 5.8s, taxonomy.

ABSTRACT

Algeria has high algal biodiversity, of great interest and is rarely exploited. Isolation of *Dunaliella* salina strain Seq Duna5.8S, from Oran Lake in Algeria, was carried out for this purpose.

Algae of the genus *Dunaliella salina* are usually used for feed, for nutritional reinforcement as a vitamin A precursor and for pharmaceuticals and fine chemicals.

The aim of this research was to identify the isolated strain by morphological and molecular taxonomy using, ARNr 5.8s, for further development in this field. In this study, we associate morphological and molecular analysis to characterize a strain Seq Duna5.8S isolated from Oran Lake. Samples of natural populations show the appearance of several green and red cells, mobile or not with different shapes in diverse stages of development, corresponding to the presence *Dunaliella salina*. Plus ARNr 5.8s analysis was to corroborate the identification and phylogenetic analyses, for clarification of the taxonomy of these microalgae.

INTRODUCTION

The genus Dunaliella was discovered for the first time in the salt marshes of Montpellier in 1838 by Felix Dunal, which demonstrated that the particular color was not due to the Physico-chemical conditions of salt marshes; but to the presence of this microorganism (Dunal. 1838), then in 1905, the name *Dunaliella* was given by Teodoresco (Teodoresco. 1905, Oren. 2005). Dunaliella belongs to the family Dunaliellaceae (phylum Chlorophyta), living in saline waters, it can adapt to waters with saturated salt concentrations (up to 5M) (Chen *et al.*2009). Interestingly some species such as *Dunaliella salina*, are known for their massive accumulation of β-carotene, which represents 10% of its dry weight.

The pigment ß-carotene is in high demand as an antioxidant and as a food coloring agent (Shaker *et al.*, 2017). Other species belonging to the genus *Dunaliella* can also accumulate ß-carotene such as *D. parva* (Lerche .1939) and *D. pseudosalina* (Massjuk.1962), but the amount is less than *D. salina* (Borowitzka & Siva, 2007, Teodoresco 1905, 1906, Hamburger 1905, Lerche. 1937, Ben-Amotzand Avorn, .1982, 1988).

According to the irradiation rate light to which it is subjected, it's apparent color changes from a predominantly green tint to a blood-red tint, it is a unicellular organism with a round or pear shape, and it moves using two flagella of equal length. Its average dimensions are 11 µm in length and 6µm in width and its volume varies between 50µm^3 and 100µm^3 (Mishra et al. 2010, Giordano. 2001; Giordano and Bowes.1997).

Dunaliella salina does not have a rigid polysaccharide wall but a thin plasma membrane elastic covered with mucous membranes (Jin and Melis.2003), which gives it an adaptation to variations in osmotic pressure (Besson. 2013). This microalga ensures photosynthesis in a hypersaline environment (Raja *et al.,* 2007, Liska *et al.*,2004). The genus Dunaliella includes 28 species (Besson .2013), however, Molecular taxonomy is an advanced and reliable method for the characterization and differentiation of morphological plastic organisms (Preetha *et al.*, 2012, Olmos .2015, Yaiche Achour *et al.*, 2018).

Identification of *Dunaliella* species/strains has already been the subject of morphological, physiological and molecular studies (Hexin *et al*. 2016, Kim.2017; Shaker.2017). In this research study, 5.8 Ribosomal RNA gene was used to identify *Dunaliella salina*, This work is original since this species was not investigated and characterized taxonomically from Algerian salt lakes before.

MATERIALS AND METHODS

Algal Sample Collection and Isolation from Salt Lack (Petit lac), Located in Oran, Algeria:

At the end of winter 2021, an algal sample was collected from an Algerian Salt Lake named "Petit Lac", situated on the southwest side of Oran (35°40'N; 0°36'O). At the sampling time, the water temperature was 15 °C, the pH of 8.81 and the salinity of 33.02 g/L. Our algae named "SeqDuna5.8s" was isolated by the monoclonal technique under an inverted microscope and was subcultured and cultured in zarrouk medium with shaking 100 rpm, 25°C +/- 1°C, Microscopic observations were made under an inverted microscope, in irradiance of 80 µmol photons mh L:D cycle L

Molecular Identification by Genetic Marker ARNr 5.8S:

The genomic DNA of SeqDuna5.8s was isolated using ADNeasy plant mini kit (Wiragen). Isolated DNA was checked by electrophoresis on 0.8% agarose gel with 1X TBE buffer and was quantified by spectrophotometer at an OD of 260nm, and frozen at -20°C until being used.

Polymerase Chain Reaction (PCR) amplifications were performed in a 25 µL reaction mixture containing 100 ng of genomic DNA, 0.6 U of *Taq* polymerase, 1X of PCR buffer, MgCl2 at 2.5 mM, dNTPs at 0.2 mM, and 0.2 µM of each specific primer.

The primers used are: the 5.8S ribosomic RNA constituting the large 60s subunit of eukaryotic ribosome.:

F: 5'-ACCTGCGGAAGGATCATTG-3'
R:5'-TCCTCCGCTTATTGATATGC-3'

PCRs program was performed as follows: 3 min of initial denaturation at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C and for 1 min, extension at 72 °C for 1 min. Reactions were completed with a 10 min final extension at 72 °C.The products of the PCR are sequenced by Sanger's Dideoxy method.

The sequences of SeqDuna5.8s were aligned with others sequences available in GenBank NCBI database via BLAST. Then, using CLUSTALW, we performed a phylogenetic analysis based on Nearest – Neighbor-Interchange and Maximum Likelihood. This was undertaken using Mega 11, and bootstrap values were retrieved from 100 replicates.

RESULTS AND DISCUSSION

Morphological Characteristics of Isolated Strain:

Using an inverted microscope, various SeqDuna5.8s algae isolates were characterised and identified as Dunaliella cells (**Fig. I**) based on morphological descriptions of Teodoresco (1906), Butcher (1959), and Massjuk (1973).

The SeqDuna5.8s algae cultured in zarrouk medium show a single-celled organism, green, motile, spherical, swollen shape because they were in the middle of cell division**.** Depending on the environmental conditions, these microalgae adopted several forms: ovoid, ellipsoidal, cylindrical, pyriform, or fusiform, the cell contained a pyrenoid (Fig.IA) surrounded by starch, and a thick mucus was observed outside the cell membrane.

According to Baas-Becking .1931, the main morphological characteristic of *Dunaliella* is the lack of a rigid polysaccharide wall (Gibbs and Duffus .1976); instead, cells were covered by an amorphous mucilaginous layer of variable thickness called a glycocalyx (Gibbs and Duffus .1976). We observed *cells* containing a cup-shaped chloroplast with a pyrenoid in the center surrounded by starch which is the storage product. The nucleus was located in the colorless anterior portion of the cells.

As reported by Borowitzka and Siva. 2007 and by Tempesta et al .2010, we also observed that some cells were more rounded, lacked flagella, and excreted a slim layer in which they divided repeatedly to form numerous green cells. This condition is named the "palmella stage". Zygospores were present (Fig.IC), they appeared spherical, with smooth walls; finally, we also observed aplanospores, particularly in old culture (Fig.IA).

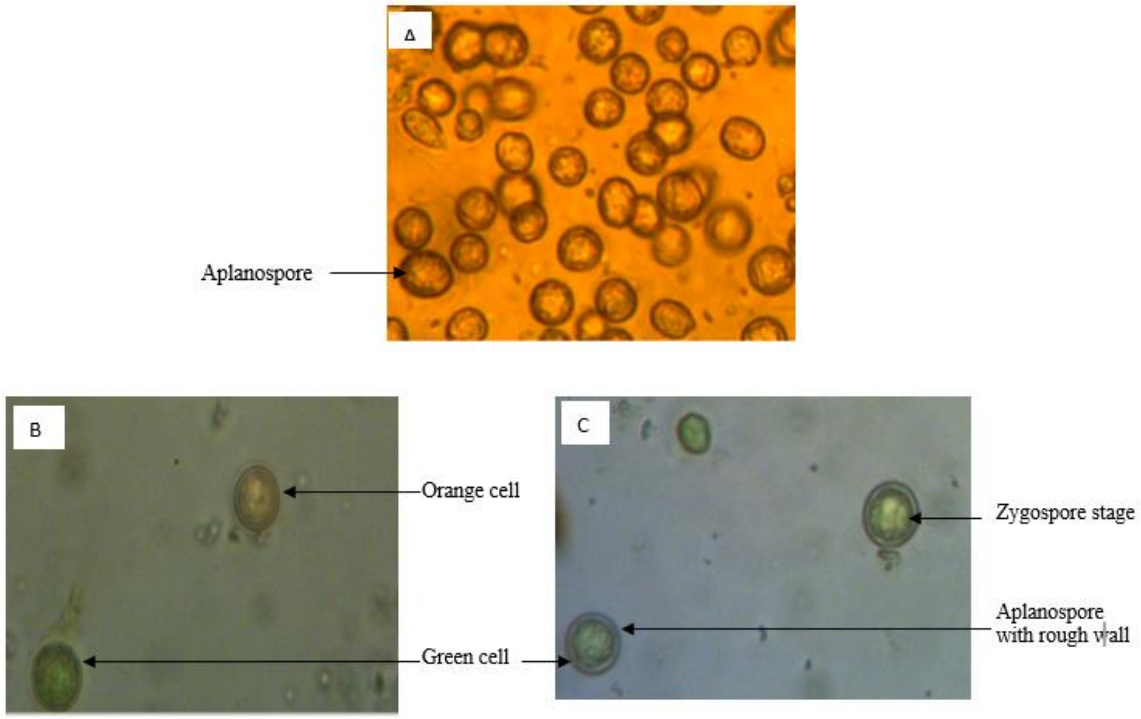

Fig. I. Inverted micrograph of Dunaliella salina. Different stages of the life cycle: (A) Agregation of different shape of cells; (B) Green and red cells; (C) Aplanospore with rough wall and Zygospora stage

Generation of the Phylogenetic Tree:

The PCR amplification of the 5.8r RNA region produced an amplicon of approximately 700pb. After sequencing, sequence alignment via BLAST, using GenBank® NCBI database, showed similarity to sequences of the gene 5.8s of *Dunaliella salina,* particularly, for the

strains: CCAP 19/39, CCAP 19/20, and CCAP 13/03, accessions numbers respectively: KJ094637.1, KJ094624, and KJ094609, with 95%, 93.94% and 93.94% of sequence identity.

In order to evaluate the phylogenetic relationships between our isolate SeqDuna5.8s algae and the other *Dunaliella species* and *D. salina* strains more accurately, ARN 5.8s gene sequence for species of reference was retrieved from GenBank® and used to perform a phylogenetic analysis, we included *Dunaliella Bioculata* as an outgroup reference. The sequences considered in the phylogenetic analysis were selected on the basis of the BLAST results. Figure (2) shows the corresponding dendrogram established by CLUSTALW. All sequences in the dendrogram were divided into three main clades A, B, and C with bootstrap values of 96, 85, and 66 respectively. Within clade A, the isolate SeqDuna5.8s algae appeared as a single group (Fig. 2 subclade E) supported by a high bootstrap value (99) and differing from the strains included in subclades D and F. The clade B included many strains from different countries: MZ079599 and MZ079592 from Russia, JQ315781.1 from Korea and MW286838.1 from Iran. The clade C included the isolates KJ094633.1 (unknown origins) and MW862500.1 (From the South of Russia).

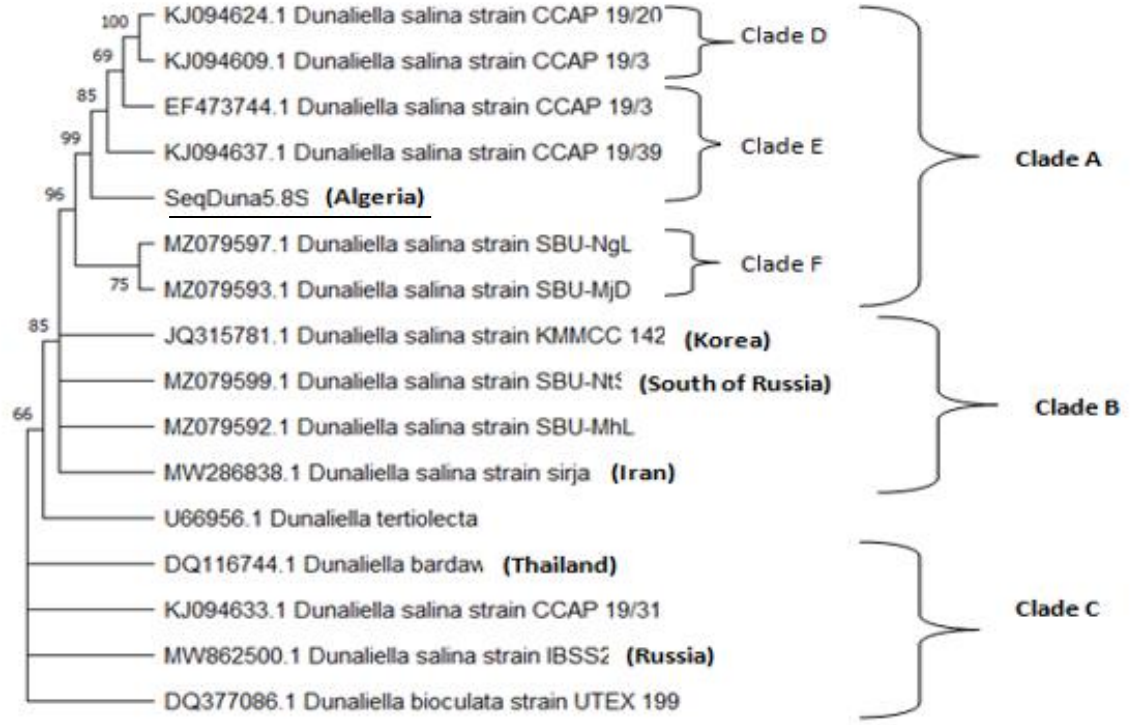

Fig. 2 : Maximum likelihood phylogenetic tree of *D.salina* based on a 5.8s rRNA gene fragment. The position *of D salina* isolated from Salt Lake of Oran (Algeria) is underlined

BLAST results indicated that the gene sequence of ARNr 5.8s matches at 95% to *Dunaliella salina* reported in GenBank NCBI. This result confirms the microscopic observations established via morphological identification. For optimal molecular identification, it would have been preferable to characterise more markers, especially molecular spacers, such as ITS regions.

Conclusion:

In this study, the strain SeqDuna5.8s isolated from a Salt Lake located in Algeria was assigned to the species *D. salina*, based on morphological and molecular characteristics. Its mass production could solve major societal and environmental problems in Algeria. Rich in proteins that can approach 40% of its dry weight under

certain growing conditions, it can provide a protein food additive for livestock feed, and as a vegetable protein substitute for animal proteins. Given its large accumulation in B carotene, it would be interesting to use it as food for salmon farming. It can also be used as a bio-remedial agent to remove heavy metals and pesticides from water.

REFERENCES

Baas-Becking, L. G. M. (1931). Salt effects on swarmer of Dunaliella viridis Toed. *The journal of general physiology*, 765-779.

Ben-Amotz , A; Katz, A. and Avron, M. (1982) : Accumulation of β-carotene in halotolerant algae: purification and characterization of β-carotene-rich globules from Dunaliella bardawil (Chlorophyceae). *Journal of Phycology*, 18: 529-537.

Ben-Amotz , A.; Lers, A. and Avron ,M. (1988): Stereoisomers of *β*-carotene and phytoene in the alga *Dunaliella bardawil*. *Journal of Plant Physiology*, 86: 1286-1291.

Besson, A. (2013). Etude multi-échelle de la récolte de Dunaliella salina Développement d'un procédé d'autofloculation -flottation de microalgues: These de doctorat INSA de Toulouse, 2013.

Borowitzka, MA. and Siva, CJ. (2007). The taxonomy of the genus Dunaliella (Chlorophyta, Dunaliellales) with emphasis on the marine and halophilic species. *Journal of Applied Phycology*, 19: 567–590

Butcher, R. W. (1959). An undescribed species of *Dunaliella* from the Cambridge collection of algae. *Journal of Hydrobiologia* , 12:249-250.

Chen, H.; Jiang, J. G. and Wu, G. H. (2009). Effects of salinity changes on the growth of Dunaliella salina and its isozyme activities of glycerol-3-phosphate dehydrogenase.*Journal of Agricultural and Food Chemistry*, 57 : 6178–82.

Dunal, F. (1838). Extrait d'un mémoire sur les algues qui colorent en rouge certaines eaux des marais salantes méditerranéennes. *Annales des sciences Naturelles Botanique*, 2 (9) :172.

Gibbs N.and Duffus, C. M. (1976). Natural protoplast *Dunaliella* as a source of protein. *Journal of Applied and environmental microbiology*, Vol. 31 (4):602-604

Giordano,M. (2001) . Interactions between C and N metabolism in Dunaliella salina cells cultured at elevated CO2 and high N concentrations. *Journal of Plant Physiology*, 158(5): 577–581.

Giordano, M. and Bowes, G. (1997). Gas exchange and C allocation in Dunaliella salina cells in response to the N source and CO2 concentration used for growth. *Journal of Plant Physiology*, 115(3): 1049–1056.

Gonzalez, M. A.; Coleman A. W.; Gomez, P. I. and Montoya, R. (2001), Phylogenetic relationship among various strains of Dunaliella (Chlorophyceace) based on nuclear ITS rDNA sequences. *Journal of Phycology*, 37: 604-611.

González, M.A.; Gómez, P.I. and Montoya R. (1999). Comparison of PCR-RFLP analysis of the ITS region with morphological criteria of various strains of Dunaliella. *Journal of Applied Phycology*,10: 573-580.

Hamburger, C. (1905): Zur Kenntnis der *Dunaliella salina* und einer Amöbe aus Salinen wasser von Cagliari, *Archiv für Protistenkunde*, 6: 111-131.

Hexin, L.; Xianggan, C.; Fazli, W.; Feng, X.; Cheng, Z., and Shiru, J. (2016). Analysis of the physiological and molecular responses of *Dunaliella salina* to acronutrient deprivation. *PLOS ONE*, 11(3): e0152226.

Jin, E. S. and Melis, A. (2003). Microalgal biotechnology: Carotenoid

production by the green algae. Biotechnology *and Bioprocess Engineering*, 8(6) :331–337.

Kim, M.; Ahn, J.; Jeon, H.and Jin, E. (2017). Development of a *Dunaliella tertiolecta* strain with increased zeaxanthin content using random mutagenesis. *Journal of Marine Drugs*, 15(6): 189.

Lerche, W. (1937). Untersuchungen über Entwicklung und Fortpflanzung in der Gattung *Dunaliella*. *Arch für Protistenkd*. 88: 236-268.

Liska, A. J; Shevchenko, A.; Pick, U. and Katz, A. (2004). Enhanced photosynthesis and redox energy production contribute to salinity tolerance in Dunaliella as revealed by homologybased proteomics. *Journal of Plant Physiology*, 136(1): 2806–2817.

Massjuk, N.P. (1972). On phylogeny and taxonomy of the genus *Dunaliella* Teod. *Journal of biology*. 29: 744-750

Mishra, A.; Kumari, K. and Jha, B. (2010). Characterization of extracellular polymeric algae Dunaliella salina. *Biotechnology and Bioprocess Engineering*, 8(6): 331–337.

Olmos Soto, J. (2015). *Dunaliella* identification using DNA fingerprinting intron-sizing method and species -specific oligonucleotides: new insights on *Dunaliella* molecular identification. In S.K. Kim (Ed.), *Handbook of Marine Microalgae* (pp. 559-568). Academic Press, 604

Oren, A. (2005). A hundred years of *Dunaliella* research: 1905-2005. *Journal of aquatic Biosystems*, 1:2

Preetha, K.; John, L. Subin, CS. and Vijayan KK (2012). Phenotypic and genetic characterisation of Dunaliella (Chlorophyta) from Indian salinas and their diversity. *Journal of Aquatic Biosystems*, 8: 27.

Raja, R.; Hemaiswarya, S .and Rengasamy, R. (2007). Exploitation of Dunaliella for betacarotene production. *Fournal of Applied Microbiology and Biotechnology*, 74(3): 517–523.

Shaker, S.; Morowvat, M.H.and Ghasemi, Y. (2017). Effects of sulfur, iron and manganese starvation on growth, ßcarotene production and lipid profile of *Dunaliella salina*. *Journal of Young Pharmacists*, 9(1), 43-46.

Tempesta, S.; Paoletti, .M and Pasqualetti ,M. (2010): Morphological and molecular identification of a strain of the unicellular green alga Dunaliella sp. Isolated from Tarquinia Salterns. *TWB, Transitional Waters Bulletin*,4 (2): (60 -70).

Teodoresco, E.C. (1905), Organization et development du *Dunaliella*, nouveau genre de Volvocacee Polyblepharidee. *Beih. Bot. Zentralblatt.Bd.18Abt*, 1:(215-232).

Teodoresco E.C. (1906), Observations morphologiques et biologiquessur le genre *Dunaliella*. *Rev. Gén. d. Bot.* T18:353-371.

Yaiche-Achour, H.; Doumandji, A.; Bouras, N.; Sabaou,N. and Assunção ,P. (2018) . Isolation, molecular identification and the Carotenogenesis Process of the microalgae Dunaliella salina Strain DunaDZ1 isolated from an Algerian Salt Lake. *Turkish journal Fisheries and aquatic sciences* 19(5): 399-407.