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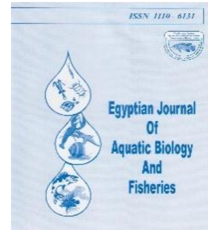

Morphometric Analysis of Sagittal Otolith in Thin-Lipped Mullet *Chelon ramada* (Risso, 1827) From Two Different Lakes, the Great Bitter and Timsah, Suez Canal, Egypt

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ABSTRACT

Mullets (Family: Mugilidae) are the most common fish species in Suez Canal lakes, contributing about 12% of the total lakes catch. They constitute a fundamental protein resource for the local communities living around the Suez Canal. The present study aimed to investigate the otolith microstructure and shape indices of ***Chelon ramada*** form the Great Bitter and Timsah Lakes. The right and left otoliths were extracted from each fish individual after measuring to the total length and weighing. The otoith's length, width, area, perimeter in addition to the shape indices were described for specimens collected from each lake separately. Results revealed that, there are significant differences in otolith shape and characters between the two fishing areas. Based on the obtained results, the otolith of *C. ramada* could be used as a useful tool to differentiate between the different localities that have different ecological parameters.

INTRODUCTION

The Suez Canal is the only canal linking the Mediterranean Sea to the Red Sea. It passes through three governorates, the Suez, Ismailia and Port Said (lying between longitudes of 32° 20' 59.99" E and 30° 27' 17.99" N), with an approximate length of 193.3km. Upon opening in 1869, the Suez Canal connected two different environments, the tropical Red Sea (an area extremely rich in species) and the sub-tropical eastern Mediterranean (an area with rather low number of species). The Suez Canal acts as a passage way to the immigrant fauna either from the Red Sea or the Mediterranean, adding to the already existing part of these fauna. Due to the importance of the Suez Canal and its lakes for both navigation and linking two ecologically different water bodies, it has been chosen for different ecological studies.

The Suez Canal has three lakes; Timsah, Great & Little Bitter Lakes. The Suez Canal lakes considered as an important fishery resource in Egypt. The fish fauna of Suez Canal lakes originates from the adjacent Mediterranean and Red seas through the Lessepsian migration after the construction of the Suez Canal. They produce high economic fish species such as mullets, tilapia, shrimps, molluscs, crab, striped piggy, seabass, seabream, cuttlefish and rabbitfish. There are about 777 sailing boats operating in Suez Canal lakes, working with several fishing methods, viz, lines, gillnets, trammel nets, crab nets, bivalve dredges and beach seine **(Eid, 2019; Mehanna *et al*., 2019; GAFRD, 2020)**.

Mullets (family: Mugilidae) are among the most common species from tropical and temperate marine coastal waters in the world, representing a fundamental protein resource for a number of human populations living in coastal areas. The family includes 26 genera and 77 species around the world **(Fishbase, 2022)**. Mullets represent about 12% of the total catch of the Suez Canal “Suez Canal, Bitter Lakes and Timsah Lakes”. Mullet's catch in Suez Canal lakes is composed mainly from *Liza carinata, Chelon ramada, L. aurata* and *Mugil cephalus*. The thin-lipped mullet *Chelon ramada* is locally known as “Tobara” along Suez Canal region, and it is mainly exploited by small fishing boats using gill and trammel net fishing methods **(Eid, 2019)**. Recently, the name *Liza ramada* was changed to *Chelon ramada*.

The morphological characteristics of fish otoliths are the most widely used tools in the species identification and comparative taxonomy of fishes due to the large size and inter-specific variability of fish otolith **(Battaglia *et al*., 2010)**. Recently, stock identification was achieved using otolith features **(Libungan *et al*., 2015; Osman *et al*., 2020; Sadeghi *et al*., 2020; Tuset *et al*., 2020; Nazir & Khan, 2021; Tuset *et al*., 2021; Yedier *et al*., 2022)**. In the present study, otolith morphometric and shape indices were investigated for *Chelon ramada* from two sites, Timsah and the Great Bitter lakes, to demonstrate if there is any differentiation or variation between *C. ramada* stock from the two fishing areas based on the otolith dimensions and shape.

MATERIAL AND METHODS

1. Study area

Timsah Lake (Fig. 1) covers an area of about 14km^2 between 30° 34' 0.01" N and 32° 16' 59.99" E near the middle of the Suez Canal at a point 80km south of Port Said city. The depth of the lake ranges between 6 and 13m. Lake Timsah receives saltwater mainly from the Suez Canal and freshwater from different sources including the Ismailia canal, rare seasonal streams and sewage outlets, creating salinity stratification in the lake’s water.

The Bitter Lakes (30°20′ N, 32°23′ E) are the largest water bodies along the length of the Suez Canal (Fig. 1), containing about 85% of the system’s water. The Great and Little Bitter Lakes, separated by narrows, are saline lakes situated between the north and south parts of the Suez Canal **(El-Bassat, 2008; Mehanna *et al*., 2019)**. The Bitter Lakes have a surface area of about 250km^2, acting as a buffer for the canal, which reduces the effect of tidal currents **(Touliabah & Taylor, 2004; Mehanna *et al*., 2019)**. Since the canal has no locks, sea water freely flows into the lakes from both the Mediterranean and the Red Seas, replacing water lost due to evaporation. The Great Bitter Lake has a surface area of about 194km^2, with depths ranging between 18 and 28m.

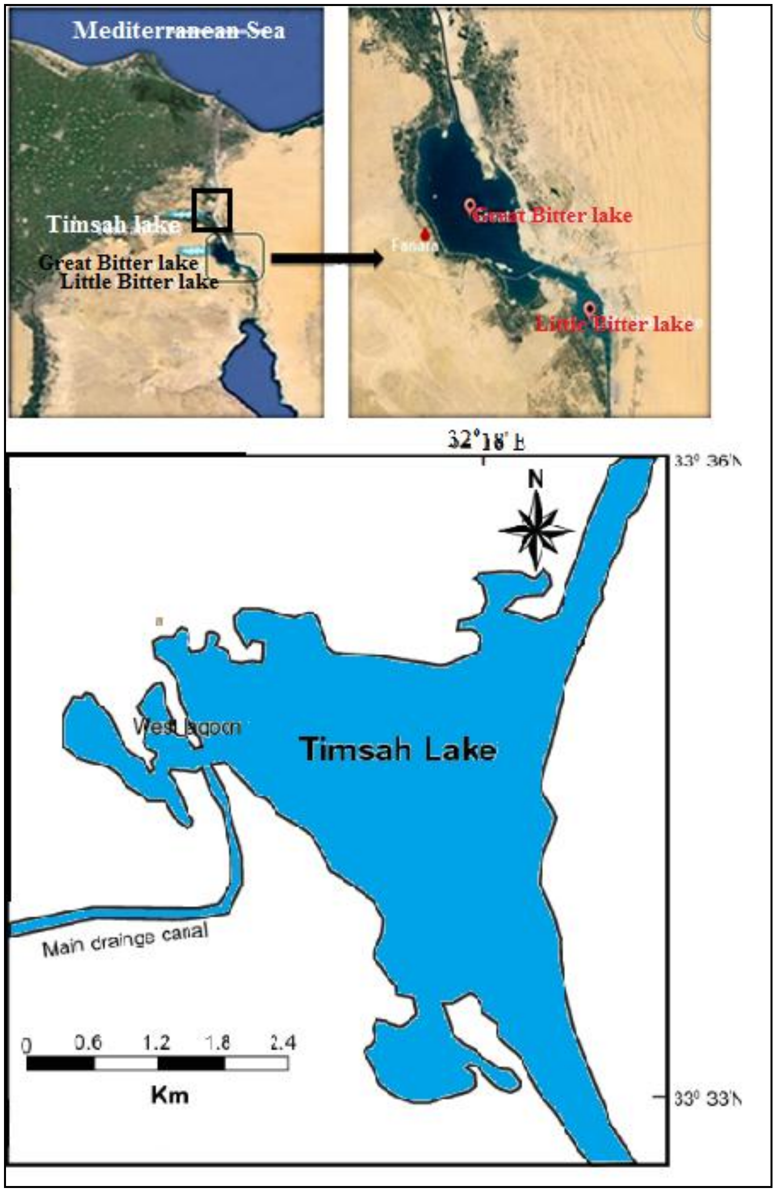

Fig. 1. Maps showing the study area: The Great Bitter Lake and Timsah Lake

2. Sampling

A total of 618 specimens of *Chelon ramada* were collected bi-monthly from the Great Bitter Lake (318 specimens) and Timsah Lake (300 specimens) during the period from January till December 2021.

3. Physical parameters

The physical parameters of the two lakes such as temperature and salinity were recorded bi-monthly for the near-surface water (50–75cm), using a portable electronic salino-meter (MC Salinity/Temperature Bridge), while the pH values were observed using pH meter (model 201/ digital pH meter).

4. Otolith investigation

Post the dissection of skull, sagittal otolith pairs were extracted, cleaned and prepared for photographing. Using the otolith's photos, the otolith length (OL), otolith hight (OH), area

(OA), perimeter (P), sulcus area (SA), otolith cauda (OCA) and otolith ostium (OST) were measured to the nearest ±0.001mm, with the use of Zeiss microscope and camera 5x1. 3.7.1 Imaging Software. The right and left otoliths were measured on two axes; the otolith length that was named as anterio-posterior axis and the otolith width as the dorso-ventral axis (Fig. 2).

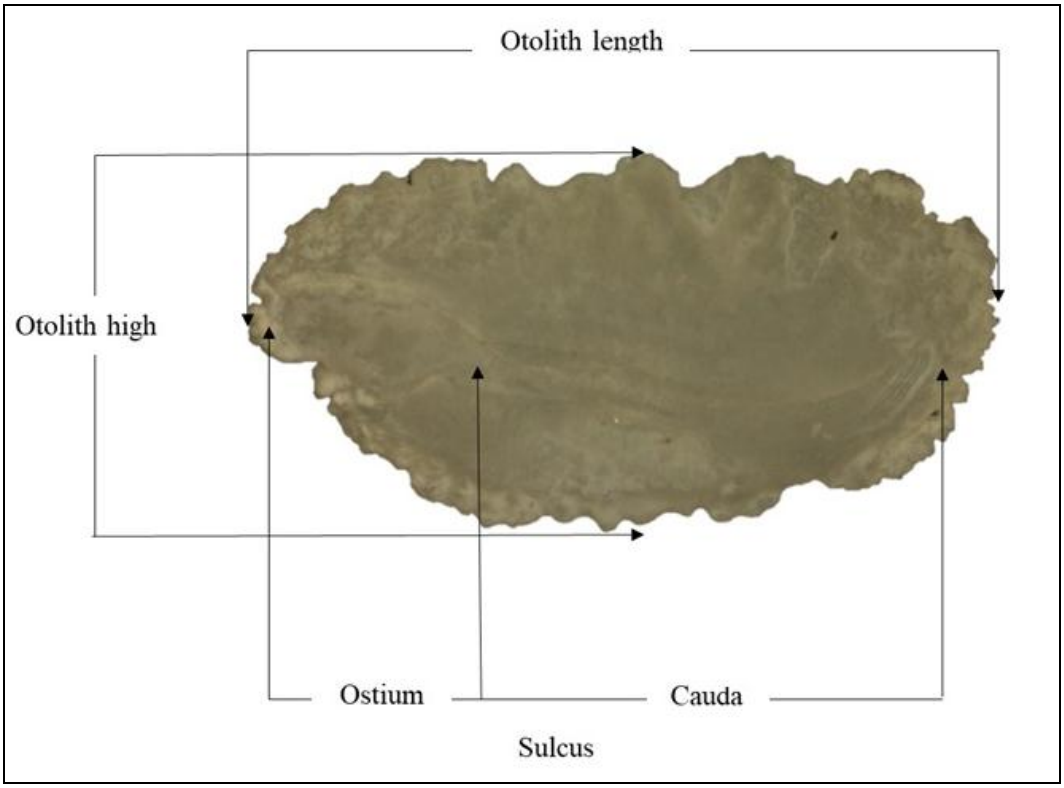

Fig. 2. Proximal view and measurement axes of the sagittal otolith of *Chelon ramada*

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The morphology of otoliths was described by two different approaches. First, the otolith shape indices were calculated, including roundness (RD), form factor (FF), circularity (C), ellipticity (E), rectangularity (R) and aspect ratio (AR) **(Tuset *et al*., 2003; Ponton, 2006)** (Table 1, Fig. 2). Second, ecomorphological indexes were calculated to identify otolith patterns, such as Edge Complexity Index (ECI), E Index (EI), R Index (RI) and S Index (SI) **(Kalff, 2002, Volpedo & Echeverría, 2003, Volpedo *et al*., 2008)**. The statistical analysis for left, right and location were examined with paired t-test to get variation between the two lakes (Great Bitter & Timsah lakes). Principal component analysis (PCA) was used to evaluate the otolith variables and PCA variables, which explained more than 1% of the variability; these variables were considered to reduce the number of dimensions without losing information. The statistical analyses were performed by excel, r studio and PAST v.2.17.

Table 1. The otolith shape morphology and formulas of otolith shape indices

Otolith parameter	Shape index	Equation
Otolith length (OL)	Aspect ratio	OH/OL
Otolith hight (OH)	Form factor (F)	4πOA/OP 2
Otolith area (OA)	Rectangularity (Rt)	OA/(OL × OH)
Otolith perimeter (OP)	Roundness (Ro)	4OA/πOL
	Ellipticity (E)	(OL − OH)/(OL+ OH)

RESULTS AND DISCUSSION

1. Physical parameters

It was found that, the temperature SST and salinity S‰ of the surface water reached its maximum during summer and early autumn, while the minimum values were recorded in winter. The SST fluctuated between 17 and 31°C in the Great Bitter Lake and between 16 and 30°C in Timsah Lake (Table 2). The high value of salinity during summer may be due to the increasing of the evaporation rate, shallowness of the lake and the high temperature during this season. The great fluctuations in salinity of Timsah Lake is attributed to the presence of freshwater sources, where the lake receives fresh water from Ismailia fresh water canal, heavy domestic, industrial and agricultural waste water from El-Mahsama drain, Abu-Gamous and untreated sewage from El-Bahtini region. Generally, the annual average of both temperature and salinity of the Great Bitter Lake is greater than that recorded in Timsah Lake, while the pH for the Great Bitter Lake is less than Timsah Lake (Table 2).

Table 2. Sample size (No), body weight (BW), total length (TL), area and environmental parameters

	Great Bitter Lake	Timsah Lake
No	318	300
TL (cm)	21.9- 37.0	21.1- 36.0
BW (g)	70.0- 320.0	80.0- 380.0
Area covers (Km^2)	194	14
Depth range (m)	18 - 28	6 – 13
SST°C	17.0- 31.0 (25.03±2.84)	16.0 – 30.0 (22 ±2.11)
pH	7.95- 8.29 (8.09±0.62)	7.85- 8.49 (8.19 ±0.14)
S‰	40.95- 44.8 (41.3±4.65)	17- 40.1 (36 ±3.44)

2. Length and weight distribution

The mullet species (family mugilidae) are widely distributed around the world and have special importance in both wild and aquaculture industries. The mullet is considered one of the most commercial fish species in Egypt, where the high quality fish meet, and their average prices are reasonable for the Egyptian. Members of family mugilidae include several species with similar morphometric characters and the same body shape, which make their external identification very difficult. Several studies deal with the biology and dynamics of mullet species **(Hosny & Hashem, 1995; El-Gammal & Mehanna, 2004; Mehanna, 2004; Mehanna, 2006; Mehanna & El-Gammal, 2007; Ahmed & El-Karamany, 2013; Eid, 2019; Mehanna *et al*., 2019a, b)**, with little attention paid to mullet taxonomy and phylogeny.

In this study, limited length range from 21.9 to 38.0 and from 21.1 to 37.0cm for the Great Bitter lake and Timsah lake, respectively (Fig. 3) were selected to study the otolith morphometrics and shape as a tool for variation detection in different localities. The weights of specimens varied between 70 and 320g in the Great Bitter Lake and from 80- 380g in Timsah Lake, reflecting the well being of the fish in lake Timsah. This may be due to the food availability and suitability of water characters in Timsah Lake.

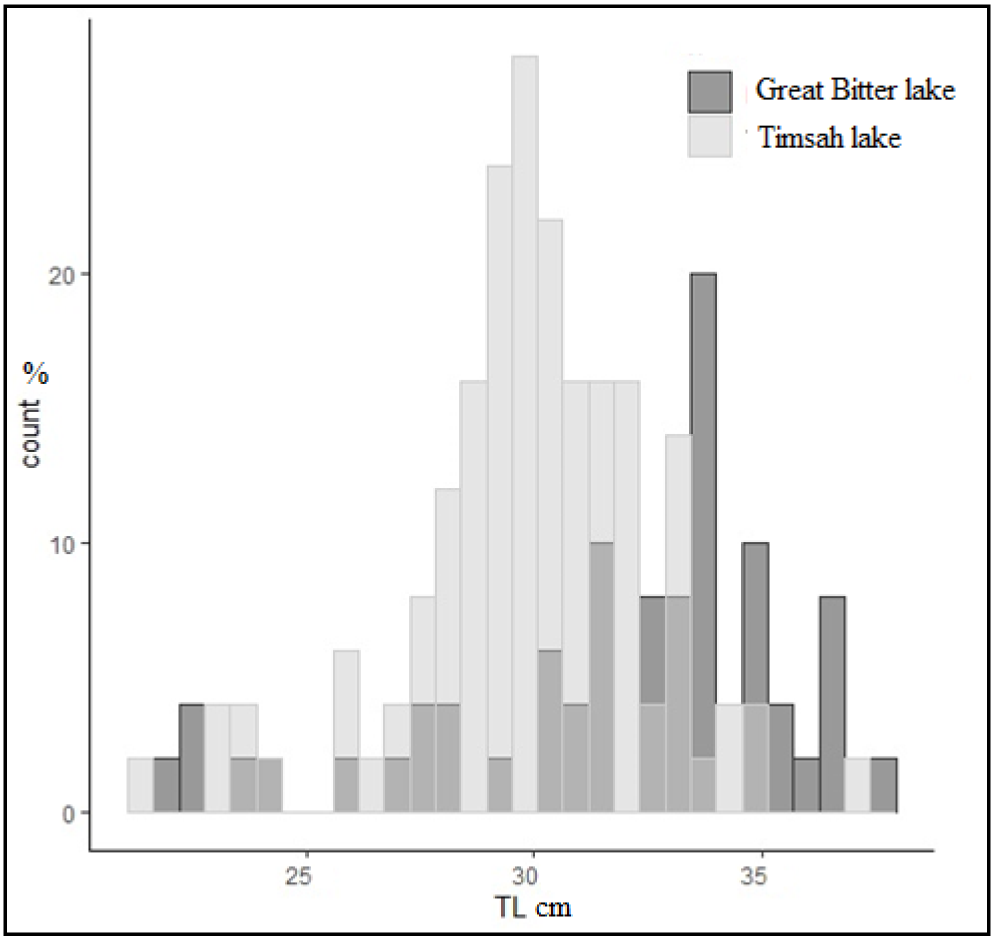

Fig. 3. Length frequency of *Chelon ramada* form the two studied lakes

3. Otolith microstructure

A total of 636 and 600 otoliths were extracted from 318 and 300 individuals of *C. ramada* sampled from the Great Bitter Lake and Timsah Lake, respectively. The shape of the otolith was illustrated as elongated close to rectangular (oval shape) for the two lakes, possessing no well-define antirostrum and rostrum; the antirostrum appeared as short and wide. The margin of otolith appeared curved and irregular; the sulcus area extended form the antirostrum and posterior, where cauda and ostium are included; cauda is long and narrow reaching to the half of otolith; the ostium area is close to a circle and short than cauda (Fig. 4).

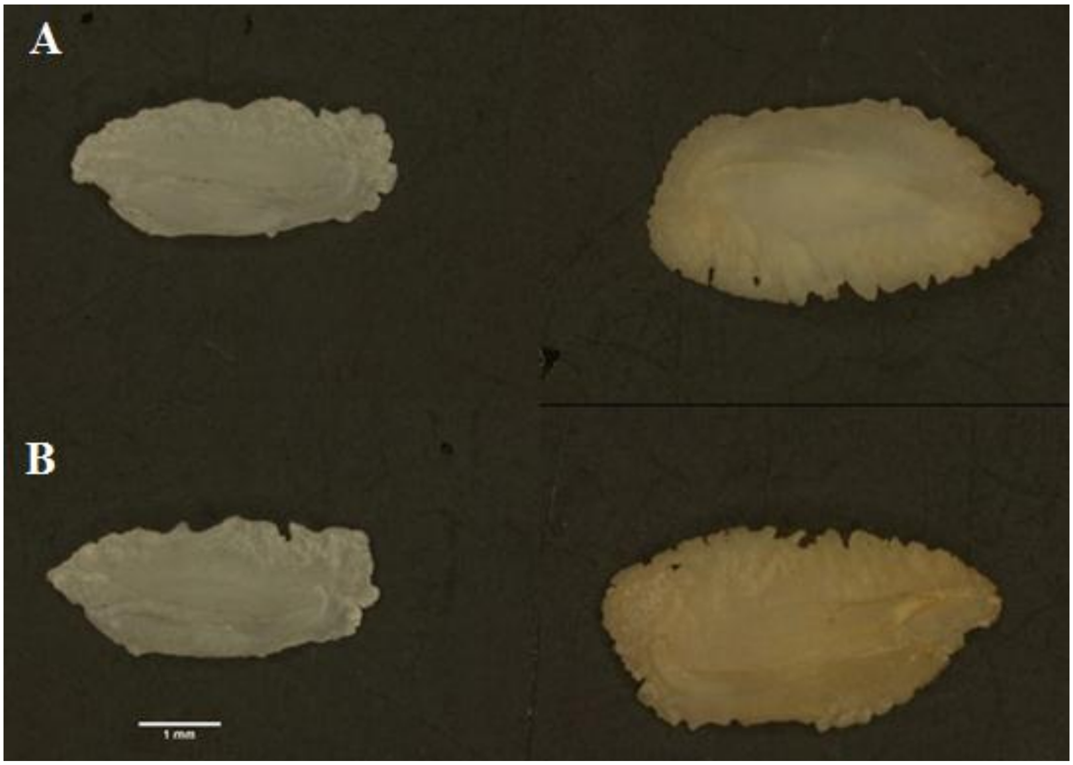

Fig. 3. The mesial surfaces of the right and left otoliths of *Chelon ramada* from (A) Great Bitter Lake (22.5cm TL and 90g in weight) and (B) Timsah Lake's population (29.5cm TL and 231g in weight)

The otolith length (OL) of left otolith varied between 3.936 and 6.155mm (average of 5.427±0.576) for the Great Bitter Lake and 4.289-6.284 (average of 5.253±0.395) for Timsah Lake, while the right otolith length ranged from 4.012 to 6.176 (average of 5.423±0.577) and from 4.178 to 6.243mm (average of 5.276±0.408) for the Great Bitter and Timsah Lakes, respectively. Various morphometric parameters of the fish were taken into consideration (Table 3). The measurements of otolith length (OL) and otolith width (OW) of both right and left otoliths were tested, and no statistically significant difference was observed ($P > 0.05$). Therefore, either the left or the right sagittal otolith can be used for the analysis. For otolith shape indices, no significant difference between otolith indices for the left and right ($P > 0.05$), except for otolith sulcus and ostium ($P < 0.05$). The paired t-test between right and left otoliths for the two locations is given in Table (3). In this study, the left sagittal otolith was used for further analysis to compare between the two lakes.

Chelon ramada (mature species) have relatively large otolith, which is more elongated in Timsah Lake than Bitter Lake (Fig. 3). The boxplot of the otolith shape indices for the two lakes was estimated and presented in Fig. (4). The minimum, maximum and median of otolith shape indices (aspect ratio, form factor, rectangularity, round and elepticity) were investigated for *C. ramada* for both fishing areas. There are significant difference between otolith length, ostium, area perimeter, aspect ratio, form factor and rectangularity, and no significant difference was observed for the other parameters, including otolith width or height, sulcus, roundness and ellipticity.

The principle component analysis was evaluated (Fig. 5) and found that, there is a variation between stocks in the two lakes. Additionally, ellipticity and roundness showed very close values for the two lakes, but the mean values of aspect ratio, form factor and rectangularity, were different in the two lakes (Fig. 5). Furthermore, the linear discriminant analysis was examined at 0.328 for the Great Bitter Lake and 0.672 for Timsah Lake (Fig. 6). The measurements' means were as follows:

	Aspect ratio	Form factor	Rectangularity	Roundness	Ellipticity
Great Bitter Lake	0.4643339	0.11987258	0.7723177	0.4567161	0.3561887
Timsah Lake	0.4814378	0.09621024	0.7554661	0.4632528	0.3508220

Recently, the morphological studies of fish otolith have gained the interest of scientists to compare fish in palaeoichthyology **(Clack , 1996; Gauldie & Jones, 2000; Homayuni *et al*., 2013; D'Iglio *et al*., 2021; Van Boeckel & Everaert, 2022)** and trophic ecology **(Durr & Gonzalez, 2002; Moreno- L´opez *et al*., 2002)**. Generally, the sagittal otoliths are the most useful structure for taxonomic variation for their large size, degree of inter-specific variation and relative ease by which the structures can be accessed **(Assis, 2003, 2004; Campana, 2004; Mille *et al*., 2015; Çöl & Yilmaz, 2022)**. In the present study, no significant difference was recorded between the left and right otolith of *C. ramada* from the two lakes; the species have the same growth for the two lakes, but the otolith length of populations from Timash Lake was larger than that in the Great Bitter Lake, indicating that Timash Lake has more suitable physical conditions than the Great Bitter lake.

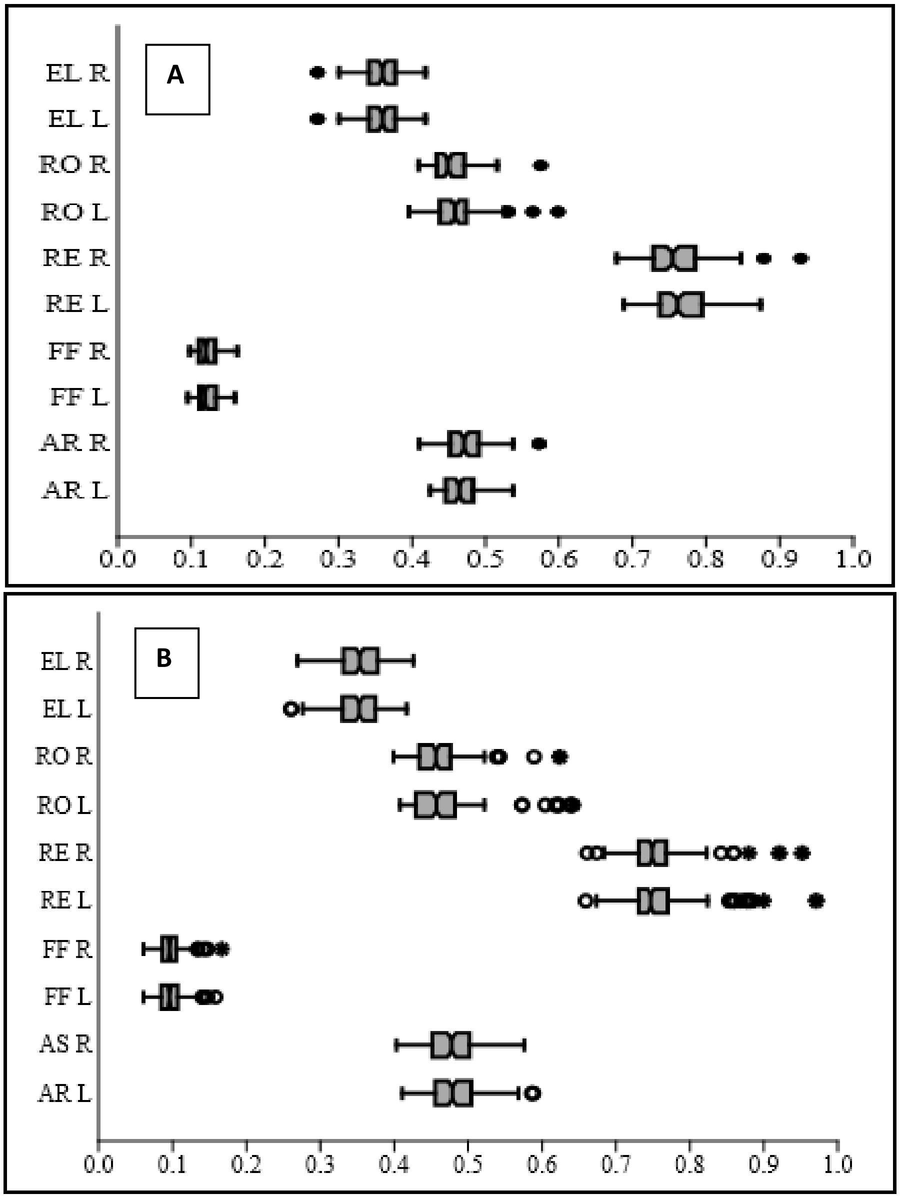

Fig. 4. Boxplot of left and right otolith shape indices for ***Chelon ramada*** from (A) Great Bitter lake and (B) Timsah lake

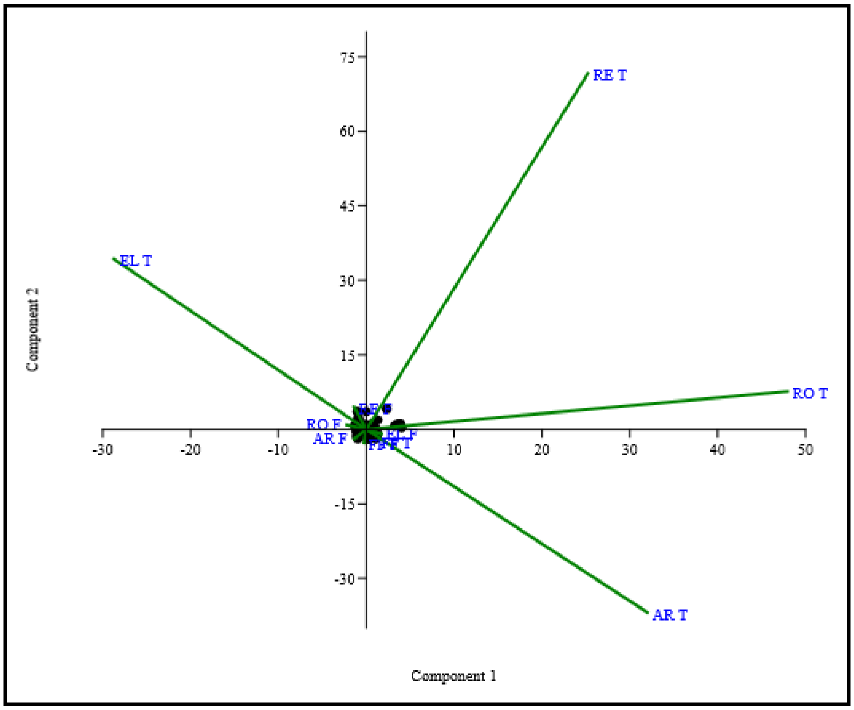

Fig. 5. Principle component analysis for ***Chelon ramada*** from the two studied lakes

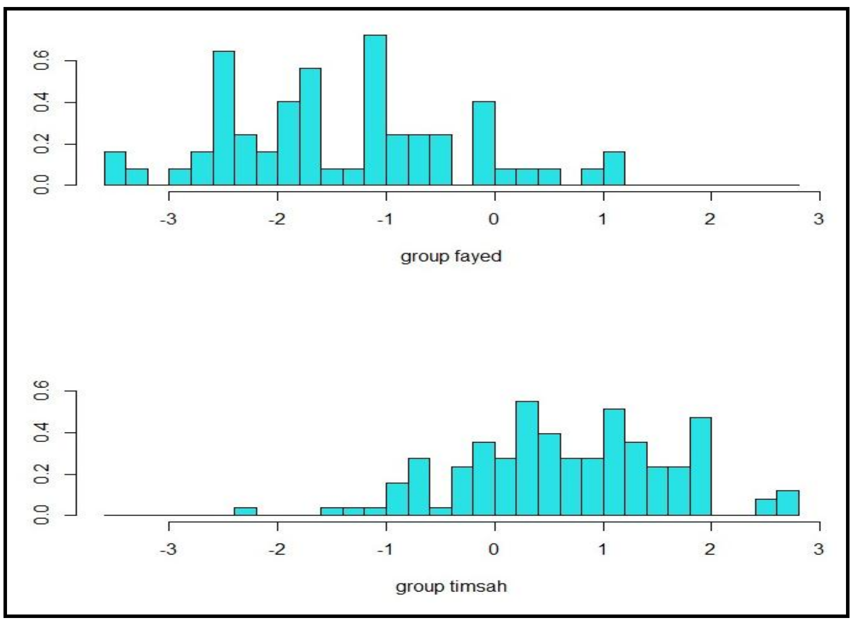

Fig. 6. linear discriminant analysis investigated for two lakes

Recently, the otolith shape indices have been used to determine the variation of otolith between or within stocks; the minimum, maximum and mean of otolith shape indices were investigated with boxplot, where otolith shape of the Great Bitter Lake was found smaller than fish otolith of Timash Lake (Table 4), thus the growth and environmental factors that influence the morphogenesis of the otolith are separated into two groups; biotic and abiotic factors viz. temperature, pH, the depth of the water in which the fish live, the nature of the substrate and salinity **(Lombarte & Leonart, 1993; Bolles & Begg, 2000; Capoccioni *et al*., 2011; Mille *et***

***al*., 2015; Holmberg *et al*., 2019; Mahe *et al*., 2019; Jawad & Mahé, 2022)**. The otolith shape indicates a significant difference between the two lakes. In the present work, sagittal otoliths were employed for their easily accessible structures, exhibiting a distinctive degree of interspecific variation in their form, weight, and growth, an explantion which is in accordance with previous studies **(Shervette & Rivera Hernández, 2022; Saltalamacchia *et al*., 2022)**.

The morphometric parameters of sagittal otolith have been used in earlier studies to identify species in other groups of teleost **(Tuset *et al*., 2003a; Ponton, 2006; Tuset *et al*., 2008; Tuset *et al*., 2020; Tuset *et al*., 2021; Jawad & Mahé, 2022; Yedier, 2022)**. This paper is the first to study the otolith characters variation for this species in the Great Bitter and Timsah Lakes, providing basic information for the use of otoliths shape and dimensions to differentiate between the different localities for the same species.

Table 3. Descriptive statistics of sagittal otolith pairs of *Chelon ramada* in the Bitter and Timsah Lakes and statistical comparisons of left and right otoliths within the population

Otolith measurement	**Bitter Lake**			**Timsah Lake**		
	Left	**Right**	***P*-value**	**Left**	**Right**	***P*-value**
OA	6.124-12.771 10.547±1.737	6.711-12.703 10.634±1.702	0.156	6.490-12.799 10.044±1.377	6.024-12.766 10.010-1.395	0.201
OP	11.681-19.901 16.602±2.009	11.569-19.901 16.616±2.066	0.392	12.042-23.831 18.211±2.103	12.040-23.831 18.178±2.103	**0.006**
OL	3.936-6.155 5.427±0.576	4.012-6.176 5.423±0.577	0.333	4.289-6.284 5.253-0.395	4.178-6.243 5.276±0.408	0.942
OW	1.880-2.907 2.520±0.247	1.801-2.901 2.567±0.260	0.923	2.098-2.923 2.502±0.182	2.098-2.900 2.492±0.177	0.159
OSU	3.246-5.519 4.490±00.549	3.226-5.519 4.474±0.540	0.286	3.554-5.925 4.536±0.493	3.558-5.922 4.526±0.495	**0.0001**
OOS	0.920-1.707 1.278±0.188	0.923-1.706 1.267±0.184	0.824	0.857-1.876 1.401±0.217	0.847-1.876 1.387±0.216	**0.024**
OCA	2.326-3.921 3.212±0.438	2.303-3.840 3.207±0.418	0.864	2.697-4.049 3.135±0.276	2.711-4.046 3.139-0.279	0.775

Table 4. Paired t-test test analysis between two locations

Measurement		Sum of squares	df	Mean square	F	Sig.
OL	Left	1.906	1	1.906	8.830	0.003
	Right	1.327	1	1.327	5.954	0.015
OH	Left	0.000	1	.000	0.007	0.931
	Right	0.194	1	0.194	4.002	0.046
OSU	Left	0.146	1	0.146	0.558	0.456
	Right	0.190	1	0.190	0.731	0.393
OOST	Left	1.074	1	1.074	24.934	0.000
	Right	1.012	1	1.012	23.919	0.000
OCUA	Left	0.427	1	0.427	2.573	0.110
	Right	0.324	1	0.324	2.001	0.158
OA	Left	17.897	1	17.897	7.885	0.005
	Right	27.503	1	27.503	12.164	0.001
OP	Left	182.784	1	182.784	42.569	0.000
	Right	172.383	1	172.383	39.429	0.000
AR	Left	0.0157	1	0.016	15.360	0.000
	Right	0.000	1	0.000	0.343	0.558
FF	Left	0.0411	1	0.041	162.450	0.000
	Right	0.046	1	0.046	182.897	0.000
RE	Left	0.013	1	0.013	6.600	0.011
	Right	0.010	1	0.010	5.101	0.025
RO	Left	0.003	1	0.003	1.691	0.194
	Right	0.002	1	0.002	1.851	0.175
EL	Left	0.002	1	0.002	1.936	0.165
	Right	0.000	1	0.000	0.341	0.560

CONCLUSION

The present study focused on the otolith morohomterics and otolith shape indices as a tool for stock identification or for dterming a comparison among the fishing areas. It provided some basic information about the otolith shape and dimensions of *C. ramada* for the first time and proved that these dimensions and shape characters could be used to differentiate between the different regions (the Great Bitter and Timsah lakes). Consequently, more detailed studies on the other species in this region or in other fishing areas in Egypt are required.

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