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Discriminant analysis as a tool to identify catfish (Ariidae) species of the excavated archaeological otoliths

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Abstract Catfish otoliths excavated from two archaeological sites in Kuwait, Sabiyah (ca. 7000 Years Before Present) and Al-Khidr, ca. 4000 YBP, were compared with those of Kuwait's modern catfish. Otoliths from Kuwait's four species of catfish, *Netuma bilineata*, *N. thalassina*, *Plicofollis dussumieri*, and *P. tenuispinis* were collected after recording total length and weight. Data recorded for both ancient and modern otoliths, including annual ring (age), weight, length and four otolith radii from transverse sections, were subject to discriminant analysis to differentiate among species and develop classification functions for otoliths. Comparisons of the results from the ancient and modern otoliths showed that most of the excavated otoliths (78% from Sabiyah and 100% from Al-Khidr) belong to the two presently dominate species *N. bilineata* and *P. tenuispinis*, indicating that ichthyofauna of Kuwait Bay may not have changed much in the past 7000 years.

Keywords Catfish · Species identification · Otolith · Discriminant analysis

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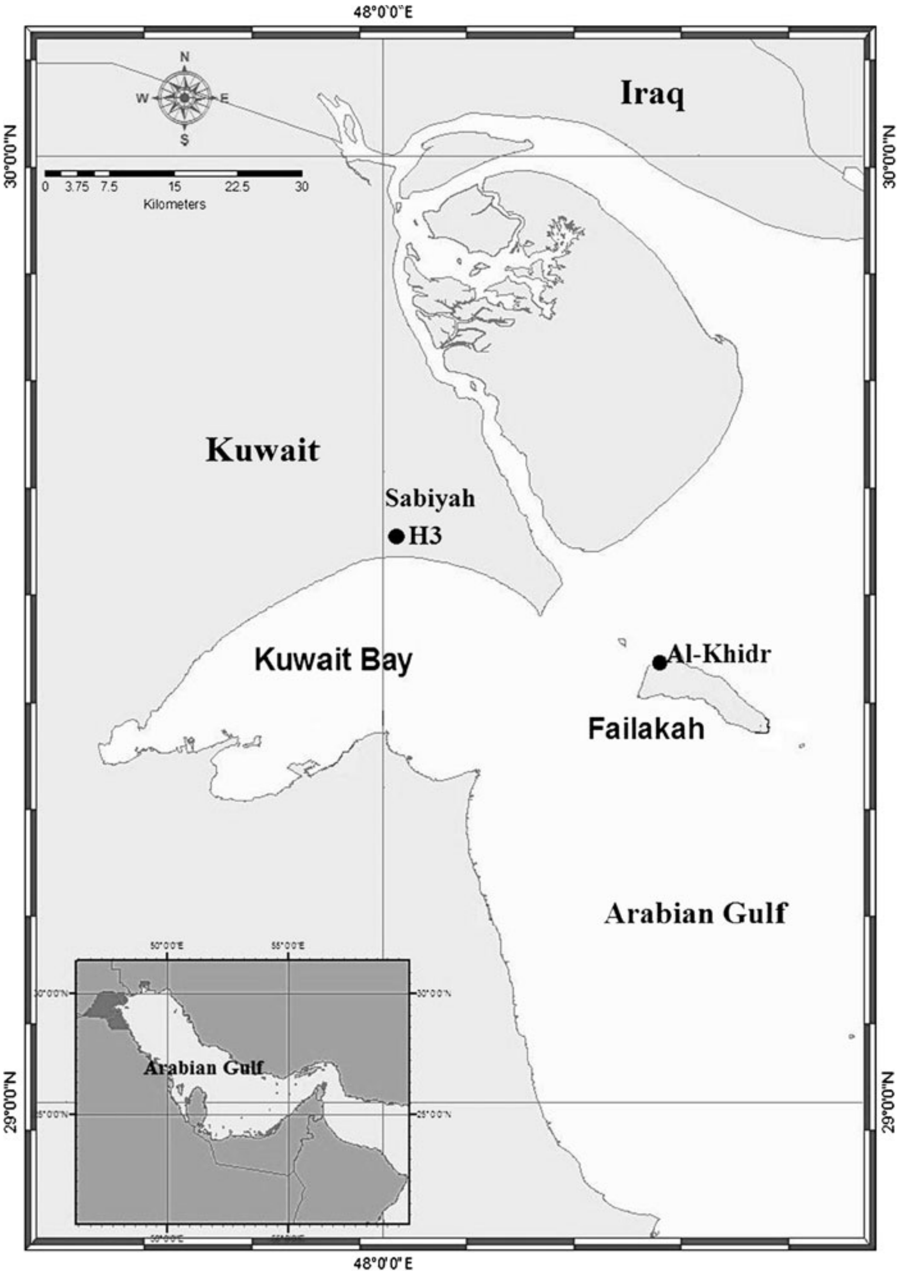
Introduction

Ubaid-related pottery of the fifth millennium BC (5000 BC) was among the preliminary findings of excavations at site H3, known as Jazirat Dubaij (Dubaij Island) in Sabiyah, an area immediately northeast of Kuwait Bay in the Arabian Gulf (Carter et al. 1999) (Fig. 1). The pottery included painted and plain shards from southern Mesopotamia, and helped archaeologists to date the site, along with carbon dating, to the Neolithic Ubaid period. The H3 site is important because of its link between civilizations found in the region; it might have been a commercial centre or a transit area for trade. Ubaid-related pottery has been found in other excavation locations in the region including the Emirate of Sharjah and southern Iraq. Shell beads found at H3 were similar to those found at Ubaid-related sites in Sharjah, and on Abu Dhabi's Dalma Island (Beech and Glover 2005) and Umm al-Qaiwain in the United Arab Emirates (Phillips 2002).

Animal remains were also among the excavated artifacts. About 70% of the animal remains belonged to a mollusc, *Lunella coronata*, which usually inhabits

Fig. 1 Kuwait map illustrating location of Sabiyah H3 site and Al-Khidr in Failakah Island



the rocky shores of the Gulf. About 95% of the recovered bones were from fish. Most of the fish bones recovered from the H3 site were caudal vertebrae belonging to several taxa: requiem sharks (Carcharhinidae), sawfish (Pristidae), rays, groupers (*Epinephelus* sp.), jacks (Carangidae), seabreams (Sparidae), emperors (*Lethrinus* sp.), flatheads (Platycephalidae), tuna (*Euthynnus affinis*) and sea catfishes (Ariidae) (Beech 2000; Beech and Al-Husaini 2005). Most of these fishes are shallow water inhabitants of sandy or sandy-muddy bottoms. Many of these could have been caught in barrier traps or gill/seine nets, while the larger fish, caught in deeper waters, are more likely to have been captured by hook and line or with basket traps. Small slabs and chunks of bituminous material remains were recovered from the site, as fragments of the waterproof coating used to cover a reed-bundle hull (Carter and Crawford 2003).

Fishing equipment discovered at the H3 site (Beech 2001) consisted of notched pebbles made from stones (probably used as net sinkers), thick pebbles that had a pecked shallow groove around them (probably used as fishing line sinkers), and bone gorges identical to bone gorges discovered in other excavated areas in the Arabian Gulf (Oman UAE).

More than 240 ancient otoliths (inner ear bones) belonging to Ariidae (catfish) species were recovered, along with about 60 otoliths from other species, such as *Epinephelus* sp., Sparidae and flat fish (Pleuronectiformes) (Beech 2001). The Ariidae ancient otoliths are well preserved and were chosen for the present investigation because of their abundance in comparison to other species.

Another important archaeological site (Al-Khidr) was recently excavated on the north-western shore of Failakah Island. Dating from the late third to early second millennium BC, the site dates from Dilmun period i.e. Bronze Age (Benediková and Barta 2009) (Fig 1). The site is rich in zoo-archaeological materials, with the majority being of ichthyological origin. Among these fish remains were vertebrae and otoliths, the majority of which were from Ariidae (Hajnalová et al. 2009). Preliminary analysis has identified at least three mammalian species (cattle, sheep, and goat) and 12 species of fish representing ten different families (Carcharhinidae, Pristidae, Ariidae, Serranidae, Carangidae, Haemulidae, Letherinidae, Sparidae, Scaridae, and Sphyraenidae). The most abundant metal finds all across the site were fish hooks (Barta et al. 2008). These hooks were with long shanks made from round or hammered rods.

Archaeozoological studies aim to shed light on human activity in these settlements, including food production, animal husbandry practices, site seasonality, and information about the local and regional palaeoenvironment.

Marginal increment analysis of ancient fish otoliths have been used in palaeontology and palaeocology to determine season of human occupation (Hales and Reitz 1992; Andrus and Crowe 2000; Higham and Horn 2000; Van Neer et al. 2002) and as a source of paleoclimate data using isotopes (Patterson et al. 1993; Patterson 1998; Andrus et al. 2002; Surge and Walker 2005).

The aim of this study was to investigate the Ariidae species whose remains were excavated from the two settlements. Four species of Ariidae are reported from the Arabian Gulf: *Netuma bilineata, N. thalassina, Plicofollis dussumieri* and *P. tenuispinis* (referred as *Arius bilineatus, A. thalassinus, A. dussumieri* and *A. tenuispinis* before, Bawazeer 1985; Al-Hassan et al. 1988). The two species, *N. bilineata* and *P. tenuispinis* inhabit shallow coastal areas including islands, while *N. thalassina* and *P. dussumieri* are larger in size and inhabit deeper and offshore waters (Al-Hassan et al. 1988). The speciation of the archaeological otoliths could provide information related to fishing activities (using hook and line) of ancient settlers at these sites and as well as their paleo-environmental distribution in the northern Arabian Gulf.

Materials and methods

Modern otoliths

Catfish samples were collected monthly from both sea surveys and fishermen at the fish markets from February 2005 through March 2007. These samples were mainly from trawls, gill nets, and *gargoors* (hemispherical wire traps) and were confined to Kuwait waters. The collected catfish samples were identified to different species based on the morphological methods described by Al-Hassan et al. (1988), Carpenter et al. (1997), and Froese and Pauly (2009). For *N. bilineata,* two different distinct types or populations were identified. Although morphologically they are one species and the L-W relationships are same, the otolith weight and fish body length correlation and the length-at-age data show two patterns. The segregation of the two groups was based on distribution patterns of otolith weight against fish length, length-at-age data and annuli widths for age 2–6. For example, the mean annulus width of age 3 type 1 is 893 μm while that for type 2 is 1312 μm. For fish of the same body size, *N. bilineata* type 1 had a larger otolith with more annual rings (older) than type 2; for fish of same age, *N. bilineata* type 1 exhibited a smaller body size. In total, 1446 individuals of *N. bilineata* [including both type 1 (Nb1) and type 2 (Nb2)], 157 *N. thalassina* (Nt), 245 *P. dussumieri* (Pd) and 2506 *P. tenuispinis* (Pt) were obtained. The sampled fishes were measured to nearest cm in total length (TL) and weighed to the nearest g for total weight.

Otoliths were removed from the specimen by cutting through the posterior region of the skull to expose otic chamber where the lapillus otoliths (the largest otolith) were dissected out, cleaned with water, dried at room temperature, and stored in paper envelopes with the appropriate data. The shapes of otolith from four species are basically the same (Fig. 2). They are circular, thick and bulbous in shape with a distinct round end rostrum (anterior) and an indistinct pseudo-rostrum (posterior). The sizes of otoliths differ depending on species groups. At a similar body size, Nb 1, Nt and Pt have relative larger otoliths than the other two groups, and Nb 1 and Pt are also much older (have more annual rings) than those of the other three groups.

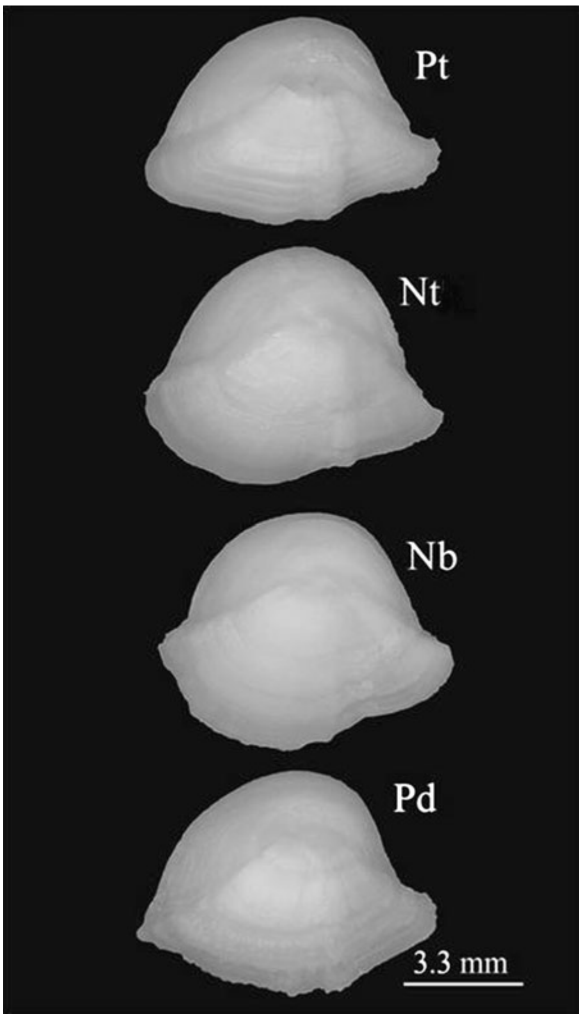


Fig. 2 Micrograph of dorsal side of lapillus otoliths of *P. tenuispinis* (Pt, TL 50 cm), *N. thalassina* (Nt, 51 cm), *N. bilineata* (Nb, TL 55 cm), and *P. dussumieri* (Pd, TL 54 cm)

Otoliths were sectioned transversely (Fig. 3) near the middle of the long axis using an Isomet saw (Buehler Inc, USA). The primordium (nucleus) is located on outer margin of the dorsal side along 1/3 of the proximal-distal axis from the distal side; the sulcus acusticus is absent from catfish lapillus otoliths.

Each catfish otolith annulus in transverse section is composed of a thin opaque zone that is most easily discerned and read under transmitted light, while the translucent zone is lighter than the opaque zone as shown in Fig. 3. The primordial regions (nucleus) are terminal in these sections and were opaque in most otoliths. Otolith accretion occurs along the axis from the otolith dorsal to the ventral side.

Otolith annual rings (fish age) were read and otolith weight (OWT), length (OL) and four other radii (R1-R4) were measured to the nearest mg and μm, respectively (Table 1, Fig. 3). The selection of radii endpoints was based on otolith features that included the primordium on the dorsal edge and end points of the proximal or distal edges. For instance, R2 is the distance between primordium and extreme point on the proximal edge, R3 is the distance between primordium and extreme point on distal side, and R4 is distance between the end points on proximal and distal edges. R1 is distance between the primordium and the furthest point on ventral edge forming a 90 degrees angle with the R3 radius. Measurement of R1, R2, R3, and R4 was performed using tools available in the imaging analysis system. In total, we obtained data from 1921 otoliths from the following groups: Nb1 (423), Nb2 (230), Nt (129) Pd (211) and Pt (928) (Table 1; Figs. 4 and 5).

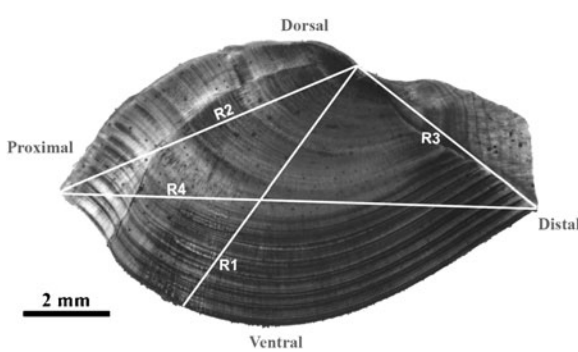


Fig. 3 Micrograph of otolith section for a 9-year*N. bilineata* sampled in September 2005 (The four radii, R1, R2, R3, and R4 measured for discriminant study are shown)

Table 1 The means with standard deviations (in parentheses) for different measures of otolith and the number of fish with valid data for different species (groups)

	OWT(g)	OL(mm)	R1(×10 μm)	R2(×10 μm)	R3(×10 μm)	R4(×10 μm)	Age (year)	N
Nb1	0.391 (0.388)	9.124 (3.110)	452.3 (184.9)	491.4 (198.9)	399.41 (138.3)	784.7 (240.0)	3.571 (3.704)	423
Nb2	1.190 (0.391)	14.391 (1.619)	788.4 (102.6)	882.2 (96.9)	531.5 (79.8)	1204.8 (124.4)	6.955 (3.139)	230
Nt	1.949 (0.754)	16.940 (2.674)	801.7 (218.3)	767.3 (173.8)	811.5 (329.7)	1457.2 (478.7)	7.770 (3.927)	129
Pd	0.833 (0.295)	13.089 (1.424)	684.7 (84.8)	752.0 (81.3)	469.1 (75.0)	1006.0 (112.7)	4.576 (2.116)	211
Pt	0.353 (0.209)	9.635 (2.138)	459.1 (119.2)	447.0 (124.8)	471.6 (156.5)	856.0 (203.2)	4.872 (3.312)	928
Total								1921

The data obtained from both male and female catfish, from different months and different fishing gears, were pooled because of the very low intra-species variation in catfishes (Simsek et al. 1990).

To reduce the effect of a fish on the shape and size of its otolith, we selected 100 fish from each group as a sub-sample, which consisted of similar size ranges between 41 and 51–89 cm depending on the specimens available, in our discriminant analysis. For Nb1 we selected all the available fishes with lengths 41 cm and above (up to length 57 cm) plus one fish of length 40 cm to obtain 100 individuals. For Nb2, Nt and Pd, we selected fish from lengths ≥41 cm until the respective number reached 100. For Pt, because the maximum length was only 51 cm (the smallest among five groups), we randomly selected 100 from 114 available specimens with lengths ≥41 cm to avoid excluding the large size fish (Figs. 4 and 5).

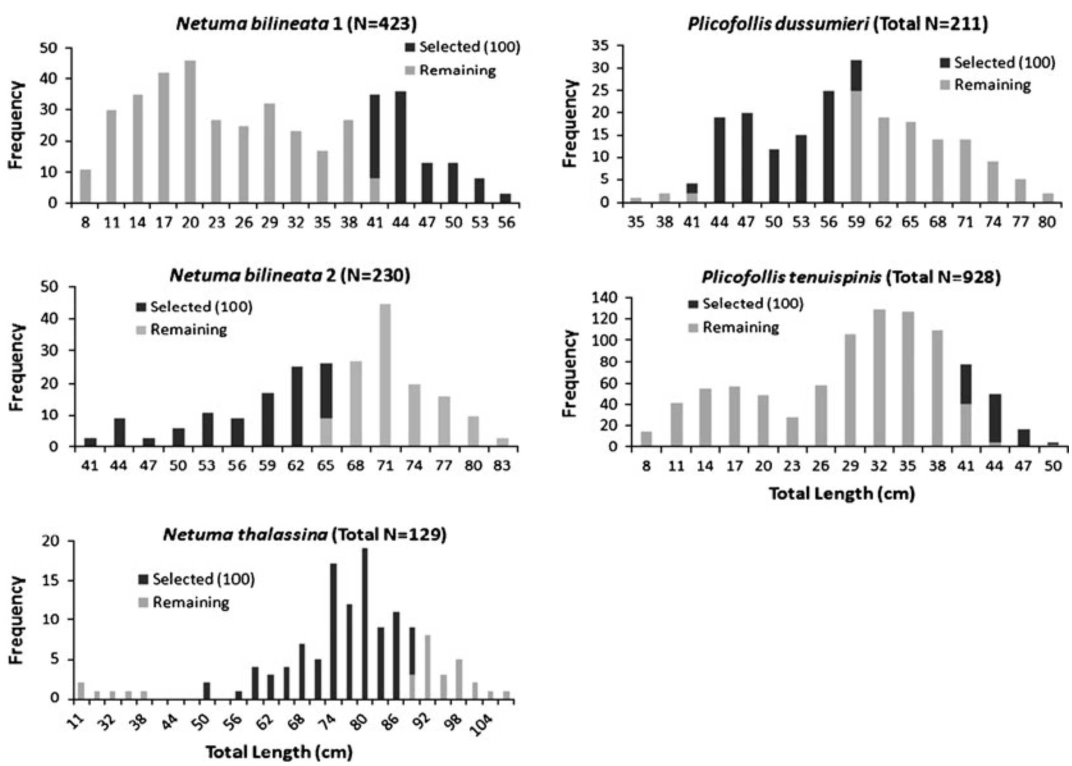


Fig. 4 Length frequencies of fishes with otolith data (Age, OWT, OL, R1, R2, R3 and R4) and the data selected in DA analysis for different catfish species. Length group 8 cm includes fishes with length 7–9 cm and the same principle applies for the other length groups

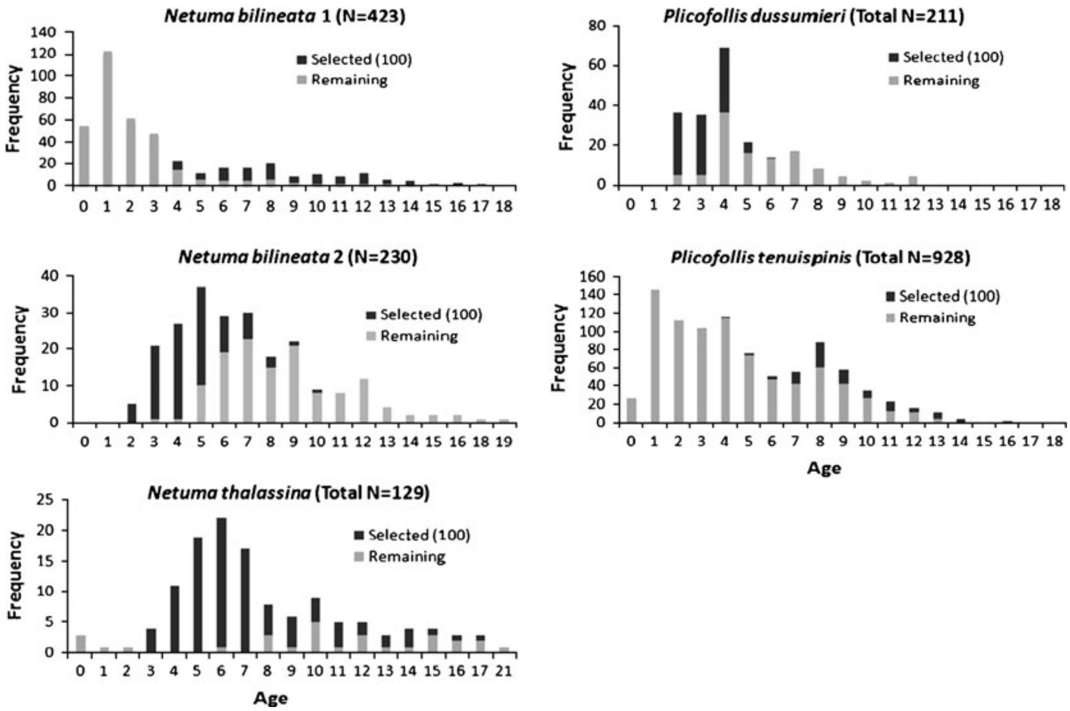


Fig. 5 Age frequencies of fishes with otolith data (Age, OWT, OL, R1, R2, R3 and R4) and the data selected in DA analysis for different catfish species

Ancient otoliths

Two hundred and six ancient otoliths from the Sabiyah H3 location and 200 otoliths from the Al-Khidr site in Failakah Island were used in our study. The ancient otoliths are basically the same as the modern ones. Otolith processing, aging and measuring techniques were the same as those applied to modern otoliths. However, the clarity and optical contrast of the sections from ancient otoliths were not as good as those of

Table 2 The correlations between otolith measurements and fish total lengths (cm)

	Nb1	Nb2	Nt	Pd	Pt
OTW	$y=0.0001x^{2.3313}$	$y=0.1119e^{0.0351x}$	$y=0.0003x^{2.045}$	$y=0.1089e^{0.0341x}$	$y=0.0003x^{2.0105}$
	$R^2=0.9668$	$R^2=0.8066$	$R^2=0.9617$	$R^2=0.9292$	$R^2=0.9681$
OL	$y=0.8915x^{0.7012}$	$y=0.8151x^{0.6863}$	$y=1.193x^{0.6113}$	$y=7.1299e^{0.0104x}$	$y=1.225x^{0.6109}$
	$R^2=0.9648$	$R^2=0.7883$	$R^2=0.951$	$R^2=0.8682$	$R^2=0.9681$
R1	$y=24.856x^{0.8703}$	$y=32.365x^{0.7629}$	$y=45.556x^{0.6548}$	$y=355.5e^{0.0112x}$	$y=41.545x^{0.7096}$
	$R^2=0.9517$	$R^2=0.734$	$R^2=0.4574$	$R^2=0.7881$	$R^2=0.9185$
R2	$y=-0.0948x^2 + 20.817x-6.2382$	$y=61.716x^{0.6358}$	$y=87.832x^{0.4958}$	$y=434.35e^{0.0094x}$	$y=46.298x^{0.6667}$
	$R^2=0.9179$	$R^2=0.7105$	$R^2=0.3914$	$R^2=0.7229$	$R^2=0.6613$
R3	$y=0.1665x^2-2.4863x+310.71$	$y=233.05e^{0.0124x}$	$y=39.242x^{0.6789}$	$y=234.02e^{0.0118x}$	$y=238.1e^{0.0207x}$
	$R^2=0.4828$	$R^2=0.585$	$R^2=0.2242$	$R^2=0.6045$	$R^2=0.2999$
R4	$y=-0.023x^2+19.195x+264.39$	$y=94.053x^{0.6097}$	$y=105.33x^{0.5944}$	$y=569.79e^{0.0097x}$	$y=151.56x^{0.5106}$
	$R^2=0.8656$	$R^2=0.7572$	$R^2=0.2731$	$R^2=0.7355$	$R^2=0.6269$
Age	$y=0.002x^{2.1371}$	$y=0.434e^{0.0407x}$	$y=0.0026x^{1.8184}$	$y=0.4849e^{0.0372x}$	$y=0.0043x^{2.0245}$
	$R^2=0.8381$	$R^2=0.6917$	$R^2=0.8159$	$R^2=0.7751$	$R^2=0.8738$

Table 3 The estimated parameters of von-Bertalanffy growth equation for different groups of catfish

	$L_\infty(cm)$	K	t_0	$W_\infty(g)$	b	N
Nb1	46.50	0.342	−0.539	1035.68	3.22	423
Nb2	77.43	0.284	−0.858	4947.24	3.49	230
Nt	90.43	0.323	0.000	7296.30	3.81	129
Pd	75.87	0.363	−0.061	4683.34	2.99	211
Pt	40.84	0.344	−0.364	704.00	2.96	928

modern ones. Nevertheless, the counting of annuli and measurement of otolith length and different radii were successfully performed except for a few specimens (not used in analysis) with peripheral edge corrosion or dark color. Ages of sectioned ancient otoliths ranged from 1 to 11 years, but the majority (87%) of the otolith samples were aged between 4 and 8 years. The otoliths with valid data numbered 139 for Sabiyah H3 and 199 for Al-Khidr.

Data analysis

Correlations between otolith measurements and fish length were fitted using Excel spreadsheet, and the parameters for von Bertalanffy growth equation of different catfish groups were established using solver in Excel.

Although DNA analysis is probably a more direct method to separate fish spices today, to extract DNA from fish otoliths, especially ancient otoliths remains difficult. Additionally, determining the species of 338 otoliths (139 from Sabiyah H3 and 199 from Al-Khidr) using DNA analysis would require special equipment and considerable funds, neither of which were readily available. An alternative method was to apply discriminant analysis together with otolith measurements including annulus, weight, length and shape. This procedure has been used for other fish species; for example, otolith growth and shape have been successfully used in discriminating Icelandic cod (*Gadus morhua* L.) populations (Petursdottir et al. 2006), Atlantic herring (*Clupea harengus*) (Burke et al. 2008) and stocks of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean (Stransky et al. 2008).

We used Discriminant Analysis (DA) in Statistica 7.0 to identify catfish species from the archaeological otoliths. All the modern otolith measures including weight, length, different radii R1, R2, R3, R4 and identified age of 100 fish from each different species (groups) were used to establish discriminant functions and classification equations. The discriminant power is described by Wilks' Lambda index for all four functions combined (0.0 denotes perfect discriminatory power and 1.0 for no discriminatory power). The classification matrix reflects how good are the classifications when we classify the known data (from which all the classification functions were developed) using the derived functions. For any otolith to be classified, scores need to be calculated using the derived five classification functions and then to compare the values of the five calculated scores. An otolith can be classified into a species group for which it has the highest classification score (S).

To further validate the DA results, all the modern otoliths including both the selected and remaining ones were also classified using the established classification functions.

Finally, the ancient otoliths were identified to species (groups) using the same classification functions.

Table 4 Discriminant function analysis summary. No. of variables in model: 7; 5 groups. Wilks' Lambda: 0.0241, Approximate F (28, 1764)=114.08, $p<0.00001$

N=500	Wilks' Lambda	Partial Lambda	F-remove 4, 489	p-level	Toler.	1-Toler. (R-Sqr.)
OWT	0.029643	0.813040	28.1117	0.000000	0.150424	0.849576
OL	0.025973	0.927933	9.4944	0.000000	0.199414	0.800586
R1	0.025626	0.940516	7.7318	0.000005	0.060174	0.939826
R2	0.027342	0.881479	16.4374	0.000000	0.114836	0.885164
R3	0.027177	0.886833	15.6001	0.000000	0.036060	0.963940
R4	0.028938	0.832845	24.5360	0.000000	0.037193	0.962808
Age	0.077334	0.311652	270.0149	0.000000	0.370406	0.629594

Table 5 Classification functions; grouping: species

Variable	Nb1 p=.20000	Nb2 p=.20000	Nt p=.20000	Pd p=.20000	Pt p=.20000
OWT	−143.265	−136.962	−117.837	−139.210	−150.775
OL	38.887	38.915	42.394	39.092	38.663
R1	0.049	0.045	0.010	0.067	0.065
R2	0.067	0.071	0.055	0.061	0.022
R3	0.062	0.038	0.081	0.066	0.040
R4	−0.054	−0.037	−0.054	−0.064	−0.033
Age	0.339	−0.907	−2.942	−1.198	1.408
Constant	−223.035	−227.599	−259.586	−219.227	−213.866

There are basically three types of DA: direct, hierarchical and stepwise. In direct DA, all the variables are analyzed simultaneously; in hierarchical DA, the order of variable entry is determined by the researcher; and in stepwise DA, statistical criteria alone determine the order of entry. Here we used stepwise DA.

Results

Correlation analysis of otolith measurements

The correlation between modern otolith measurement and fish length showed different patterns for different species (Table 2). Nt had a single power correlation with fish length for all otolith measurements although the correlations were relatively poor for R1-R4. Pd had a similar logarithmic correlation for all otolith measurements, while Nb1, Nb 2 and Pt had more than one type of correlation between their otolith measurements and fish length.

Von Bertalanffy growth parameters for different modern catfish groups

The fitted von Bertalanffy growth parameters showed different growth patterns between different catfish groups (Table 3). Nt had the largest length at infinite age (L_∞), followed by Nb 2 and Pd. Nb 1 and Pt had much smaller L_∞ than the other three groups. The growth rates (K) also differed among groups. The Pd had the largest K value while Nb 2 had the smallest with other three groups between them.

DA general

The discriminant analysis results showed all seven variables (measures) were included in the models to separate the five groups of modern otoliths. The Wilks' Lambda for all four functions combined was relatively low (0.0241) and the relative approximate F was high [$F(28, 1764)=114.08$ ($p<0.00001$)] indicating high discriminant power.

The Wilks' Partial Lambda value showed that age contributed the most discriminatory power among groups (Partial Lambda value is the smallest for age), followed by OWT, R4, R2, R3, OL and R1 (Table 4). The tolerance value (Toler.) showed that none of the variables is redundant (Toler.<=0.01) and should be excluded.

Canonical analysis

The detailed Canonical Analysis is included in the Appendix.

Table 6 Classification matrix based on the selected sub-samples. Rows: observed classifications. Columns: predicted classifications

Group	Percent Correct	Nb1 p=0.2000	Nb2 p=0.2000	Nt p=0.2000	Pd p=0.2000	Pt p=0.2000	Total
Nb1	70.00	70	15	0	9	6	100
Nb2	86.00	3	86	0	11	0	100
Nt	97.00	0	1	97	2	0	100
Pd	89.00	0	11	0	89	0	100
Pt	84.00	14	0	0	2	84	100
Total	85.20	87	113	97	113	90	500

Classification

The classification functions (not the discriminant functions) were computed for each modern catfish species (group) and used directly to classify new cases (Table 5). For example, the classification function for species Nb1 is:

$$\begin{aligned} Nb1 = {} & 143.265OWT + 38.887OL + 0.049R1 \\ & + 0.067R2 + 0.062R3 - 0.054R4 \\ & + 0.339Age - 223.035 \end{aligned}$$

The classification functions for the other four groups are similar but with different parameters for each variable as listed in Table 5.

The classification matrix comparing the predicted classifications obtained from classification functions with known data (the selected 100 fish) showed that Nt had the highest percent correct (97%), and Nb1 the lowest (70%), the overall mean was 85.2% (Table 6).

The classifications of all the modern otoliths including both the selected and remaining ones using the developed classification functions (Table 7) showed that the percent correct decreased in comparison with those of selected otoliths only, which indicates the possible effect from fish size. The extremely low percent correct (20%) for Nb 1 was probably caused by large number of small size fish of age 0–1 in the sample. However, since there were no year 0 and few year 1 otoliths among the ancient otoliths, this problem may not affect the classification of the ancient otoliths.

The classification of the ancient otoliths from Sabiyah H3 showed that two-thirds of the otoliths belonged to either Pt (36%) or Nb 1(35%). Only one-third of the ancient otoliths belonged to the other three groups. Almost all the ancient otoliths (196 out of 199) from the Al-Khidr site belong to Pt, with only three cases being assigned to the Nb 1 population (Table 8).

Discussion

The types and coefficients of the correlations between modern otolith measurements and fish length vary among fish groups. Different types of correlation reflect the differences in otolith growth (size, shape and increments) associated with growth of fish length among different fish groups. These differences will help DA in separating fish groups. At similar body lengths, otolith size and the annual rings on otolith (fish age) are quite different among fish groups. Nb 1, Nt and Pt have larger otoliths than those of the other two groups (Nb 2 and Pd), and Nb 1 and Pt also have more annual rings (older) than those of the other three groups. These differences can also be seen when one back calculates the otolith measurements of a fish with same body lengths but from different groups using the correlations in Table 2. Our DA results show that among the variables tested, age contributed the most to the discriminant power, followed by otolith weight (OWT) and otolith width (R4), which matches the results from the correlation analysis that age and otolith size are quite different among different fish groups.

DA involving all the samples was also conducted before using the selected similar-length data, using the selected similar-length data increased the values of the Wilks' Lambda for all four functions combined

Table 7 Validation of the classification functions using all samples. Rows: Observed classifications. Columns: Predicted classifications

Group	Percent Correct	Nb1	Nb2	Nt	Pd	Pt	Total
Nb1	19.39	84	23	0	189	127	423
Nb2	82.17	25	187	3	13	0	230
Nt	93.85	0	2	122	5	1	129
Pd	71.09	11	49	0	150	1	211
Pt	75.32	54	10	0	165	699	928
Total	64.65	174	271	125	522	828	1921

Table 8 Classification results (%) for the excavated ancient otoliths, the numbers in the parentheses are the numbers of otoliths classified into that group

Species	Sabiyah H3 (N=139)	Al-Khidr (N=199)
Netuma bilineata type 1	34.53 (48)	1.5(3)
N. bilineata type 2	7.91 (11)	0
N. thalassina	2.16 (3)	0
Plicofollis dussumieri	19.42(27)	0
P. tenuispinis	35.97(50)	98.5 (196)

from 0.0668 to 0.0241 reflecting a remarkable increase of the discriminant power. This indicates that the size differences in fish of different groups will reduce (not increase) the discriminant power.

Another possible way to increase the discriminant power is to select similar fish-age groups, not fish-size ranges, of modern otoliths with those from ancient otoliths. Although selection of similar fish-age groups will certainly increase the size differences of fishes. It will reduce the age variations among different groups, and might increase the precision of classification.

The fitted von Bertalanffy growth parameters for different groups of catfish also support the separations between groups. Nt has the largest length at infinite age (L_∞), followed by Nb 2 and Pd, while Nb 1 and Pt have much smaller L_∞.

The Ariidae otoliths were the most dominant osteological remains in the both excavated sites, which indicates that these species were more commonly exploited than other species. Species classification of ancient otoliths from the Sabiyah H3 site indicated that 78.4% of these otoliths belong to two species (*N. bilineata* and *P. tenuispinis*), while 99% of otoliths from the Al-Khidr Bronze-age site are *P. tenuispinis*. Both *N.bilineata* and *P. tenuispinis* are expected in fish catches from Kuwait Bay and around Failakah Island (AL-Hassan et al. 1988; Carpenter et al. 1997). Ariidae species can be caught easily by hook and line as well as by other fishing gears. We believe that fishing was carried out mostly by hook and line in the shallow waters of these two sites, but fishing in deeper areas most probably also took place, as inferred from low number of otoliths of other Arridae species that reside in deeper water, i.e., *N. thalassina* and *P. dussumieri.* The zooarchaeological studies of fish bone assemblages from Ubaid sites (H3 in Kuwait, Dosariyah in Saudi Arabia, and Dalma Island and Umm al-Qaiwain in the U.A.E.) clearly demonstrate that fishing did occur in deeper offshore waters for larger pelagic species such as sharks, tuna, scads, trevallies, snappers, breams, sawfish and groupers (Beech 2001). A preliminary study on time of annuli deposition in Ariidae otoliths excavated from Al-Khidr, Failakah Island suggests that fishing at this site took place mostly during the spring, between April and June (Al-Husaini et al. 2008).

The species identifications of the excavated ancient otoliths possibly revealed a very long history (7000 year) of exploitation of *P. tenuispinis* and *N. bilineata* as the dominant catfish species in the Arabian Gulf region.

In conclusion, the high discriminant power indicated that all seven variables used in the DA were important in separating the five groups of modern catfish species. The average percentage correct of 85% was relatively high and justified the methodology applied to the ancient otoliths in this study. In addition, the DA methodology applied here could be used as a validation tool for species morphometrics and DNA analysis. However, geometric morphometry (shape analysis) would be desirable in future research because it might increase the discriminant power and precision of species classifications.

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Appendix

Canonical analysis

Because there were five groups of otoliths, four discriminant functions (roots) could be developed for differentiation between groups. The Chi-Square Tests with successive roots removed showed that all four discriminant functions were statistically significant (Table 9). The standardized coefficients for canonical variables (Table 10) showed that the first discriminant function (root 1) is weighted most heavily by age (with the largest absolute value), and second most

Table 9 Chi-square tests with successive roots removed

Roots removed	Eigenvalue	Canonical R	Wilks' Lambda	Chi-Sqr.	df	p-level
0	6.786378	0.933579	0.024101	1836.667	28	0.0000
1	2.247465	0.831906	0.187662	824.846	18	0.0000
2	0.387589	0.528512	0.609424	244.153	10	0.0000
3	0.182550	0.392899	0.845630	82.663	4	0.0000

Table 10 Standardized coefficients for canonical variables

Variable	Root1	Root2	Root3	Root4
OWT	1.19086	−0.087205	−0.07086	0.03745
OL	0.56743	0.319664	0.16394	−0.12834
R1	−0.74460	−0.323257	1.24002	−0.14998
R2	0.21479	−0.863744	−1.01222	−1.10758
R3	0.75438	0.182335	1.02662	−3.88080
R4	−0.39268	0.576225	−1.64984	4.67221
Age	−1.26644	0.630854	−0.81331	0.04341
Eigenvalue	6.78638	2.247465	0.38759	0.18255
Cum.Prop	0.70662	0.940635	0.98099	1.00000

Table 11 Means of canonical variables

Group	Root1	Root2	Root3	Root4
Nb1	−1.35600	0.09762	−0.727608	−0.650626
Nb2	−0.02704	−1.75499	−0.577233	0.561936
Nt	4.77370	1.15808	0.045434	0.000327
Pd	−0.42714	−1.56276	0.990837	−0.239218
Pt	−2.96352	2.06205	0.268570	0.327581

heavily by OWT, and followed by R3, R1, OL, R4 and R2. The second discriminant function (root 2) is weighted most heavily by R2, and secondly by age, followed in decreasing order by R4, R1, OL, R3, and OWT. The third discriminant function (root 3) is most heavily weighted by R4, followed in decreasing order by R1, R3, R2, age, OL and OWT. The Eigen-values and cumulated proportions (Cum.Prop) in Table 10 showed that the first function (with a value of 6.79) accounts for approximately 70% of the discriminatory power, the first and second functions together explain 94% of the discriminatory power, and the first three functions together explain 98% of the discriminatory power. The fourth discriminant function (root 4) only accounts for 2% of the discriminatory power.

The means of canonical variables (Table 11) and the scatter plots for the each two selected discriminant functions (Figs. 6 and 7) separate the different groups. Canonical variable means for root 1 ranged from −2.96 to 4.77, and no two means are very close (Table 11). Otoliths of Nt and Pt fall far to the right and left, respectively, and bracket otoliths of the other three groups on the plots of canonical scores for root 1 vs. root 2 (Fig. 6) and for root 1 vs. root 3 (Fig. 7). Thus, the first discriminant function (root 1) separates species Nt from Pt, and Nt and Pt from the other three

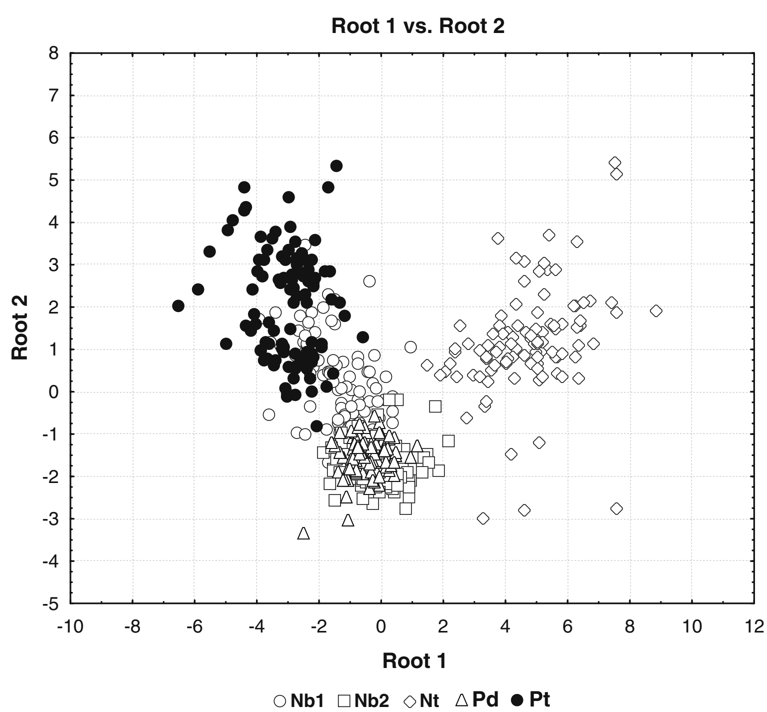

Fig. 6 The scatter plot of canonical scores for Root1 vs. Root 2

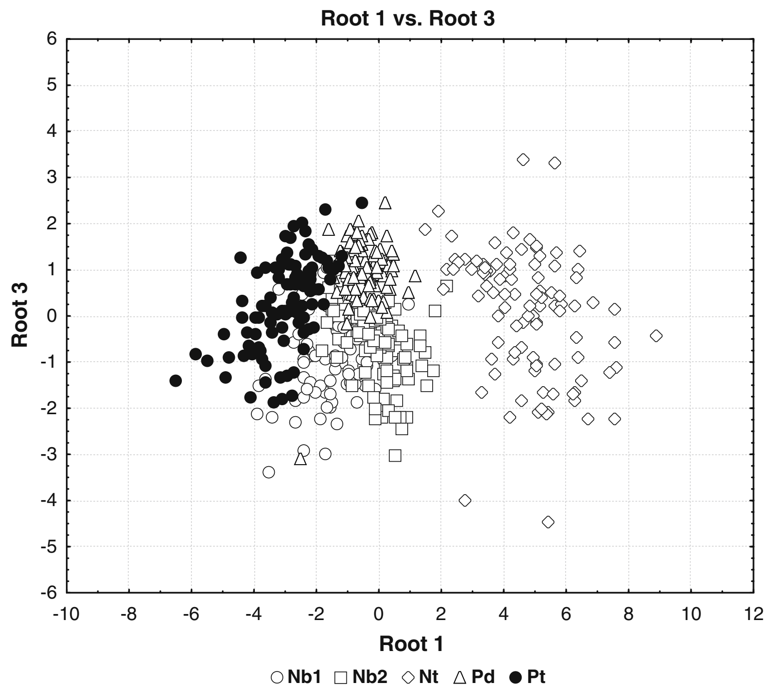

Fig. 7 The scatter plot of canonical scores for Root1 vs. Root 3

groups. The second function (root 2) provides some discrimination between Nb1 and the other two groups (Nb2 and Pd) (Fig. 6). The root 2 mean for Nb1 is slightly positive, whereas those for Nb2 and Pd are obviously negative (Table 11; Fig. 6). Root 3 discriminates between Nb2 (plots relative down with negative mean) and Pd (plots relative up with positive mean) (Table 11; Fig. 7).

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