SHORT COMMUNICATION

Incremental growth and mineralogy of Pannonian (Late Miocene) sciaenid otoliths: paleoecological implications

ZOLTÁN KERN^{1,2,™}, MIKLÓS KÁZMÉR¹, MARIANN BOSNAKOFF³, TAMÁS VÁCZI⁴, BERNADETT BAJNÓCZI² and LAJOS KATONA⁵

¹Department of Palaeontology, Eötvös Loránd University, Pázmány Péter sétány 1/c, H-1117 Budapest, Hungary; mkazmer@gmail.com ²²Division of Climate and Environmental Physics, University of Bern, Sidlerstrasse 5, CH-3012 Bern, Switzerland; kern@geochem.hu

²MTA CsFK Institute for Geological and Geochemical Research, Hungarian Academy of Sciences, Budapest, Hungary

³Hungarian Natural History Museum, Budapest, Hungary

⁴Department of Mineralogy, Eötvös Loránd University, Budapest, Hungary ⁵Natural History Museum of Bakony Mountains, Zirc, Hungary

(Manuscript received September 14, 2011; accepted in revised form January 4, 2012)

Abstract: Ontogenetic age and body dimensions were studied on three extremely well-preserved sciaenid fish otoliths from sublittoral marls of Lake Pannon from Doba, Bakony Mts, Hungary. Macroscopic and microscopic observations offered clear evidence for the preservation of the genuine structural characteristics, for instance the bipartite incremental features. Ontogenetic ages were assigned for the three specimens as 16, 7 and 6 years by counting the annuli of the sagittae. Analytical results prove that the original aragonitic mineralogy has been preserved making them, and probably other Late Miocene teleost fossils, suitable for future microchemical analysis to reconstruct the past physicochemical environment.

Key words: Lake Pannon, paleoecology, littoral environment, sagitta, cathodoluminescence, Raman microspectroscopy, aragonite.

Introduction

Lake Pannon, a long-lived, giant lake, was a Late Miocene successor of the disintegrated Paratethys Sea (Kázmér 1990). Extensive freshwater discharge by the paleo-Danube and paleo-Tisza rivers into the Pannonian basins (Kuhlemann et al. 2002) altered the composition of the water-body, reducing the marine-derived fauna of the Late Sarmatian brackish-water sea into a fully endemic, lacustrine fauna. While the mollusc fauna is well-documented (e.g. Müller et al. 1999 and references therein), the fish fauna is poorly known. Mostly otoconia (mineral material from the inner ear of any vertebrate; see Carlström 1963) from sciaenids have been found relatively frequently (e.g. Brzobohatý & Pana 1985; Bosnakoff 2008).

The endolymphatic organ of Sciaenidae, like in other teleost fishes, contains three pairs of small carbonate stones, so-called otoliths. The largest of them are called sagittae, located in the sacculus of the membranous labyrinth. Although both incremental (e.g. Woydack & Morales-Nin 2001) and microchemical information (e.g. Carpenter et al. 2003) of fossil otoliths proved to provide unique information about the coeval paleoenvironment, these parameters have never been studied on the Pannonian otoliths. They are independent proxies for the temperature and salinity of Lake Pannon. Water temperature and salinity estimates show a wide range of fluctuations during its history. A bottom-water temperature of ~15 °C and variable salinities (20-32 ‰) are estimated for the Early Sarmatian sea, while temperature and salinity are estimated to have fluctuated from 15 °C to 21 °C

and from 15 % to 43 %, respectively in the Late Sarmatian sea (Tóth et al. 2010). These values are in range with estimates from the composition of microfauna and molluscs. As Lake Pannon hosted many endemic species (Müller et al. 1999; Geary et al. 2000) the isolation precludes direct estimation of salinity from faunal composition or from oxygen and carbon isotope studies of bivalve shells (Geary et al. 1989; Mátyás et al. 1996). The Late Sarmatian-Pannonian samples have higher ratios of heavy isotopes (-2.11 % for δ^{18} O, and 1.27 ‰ for δ^{13} C) than do the succeeding Pontian Stage samples (-4.16 ‰ and -2.22 ‰, respectively). This shift was interpreted as indicative of a basinwide drop in salinity (Geary et al. 1989).

In addition, the isotope data clearly indicate that Lake Pannon was not a huge, closed freshwater lake. Assuming a riverine outlet somewhere along the southern margin, salinity would have been lost and full freshwater conditions appear in a couple of tens of thousands of years. In contrast, a closed basin with no outflow needs evaporation of all inflowing water and rainfall - impossible under present-day climatic conditions (Leeder 2007).

Three otolith samples have been selected for the purpose of this pilot study. The scope was to investigate the preservation of original incremental and mineralogical structure and decide if the chemical signature remained unaffected by post-depositional processes. The preservation of the genuine microchemistry of the fossil otoliths is a substantial prerequisite for any future paleoenvironmental or paleoecological application.

Materials and methods

Fossil otolith samples

Sagittal otoliths were collected from the upper 0.9 m of a 2.6 m deep trench profile dug in a Late Miocene sequence at Doba village, Bakony Hills, Hungary (Fig. 1). The age of the fossiliferous littoral marl layer of the *Lymnocardium ponticum* Zone is estimated as 8.8–9.2 Ma (Magyar et al. 2007). Twenty-nine otoliths were found after sieving (mesh size: 500 micrometers) of ca. 300 kg bulk material. Most of the samples (28) were determined as *Umbrina subcirrhosa* Schubert, 1902 based on the morphological characteristics.



Fig. 1. Geographical and geological setting of the study site. Star indicates the location of the studied Doba outcrop. Major geological units (after Cziczer et al. 2009): 1 — pre-Late Miocene basement, 2 — sublittoral deposits of Lake Pannon, 3 — littoral to deltaic deposits of Lake Pannon and overlying fluvial sediments, 4 — Upper Miocene-Pliocene volcanics. Inset map shows the location (black square) on a map of Europe.



Fig. 2. The studied Pannonian sagittal otoliths, *Umbrina* cf. *subcirrhosa* Schubert, 1902. Top row shows the internal view with the characteristic sulcus acusticus. The length of each specimen is indicated. The bottom row shows the polished sections under reflected light. Tip of the arrow points to the spot of the SEM image in Fig. 3b. Rectangles help comparison with the CL images in Fig. 3a.

For further details about the material, the reader is referred to Bosnakoff & Katona (in print).

Three samples have been selected from the collection to represent large (UC1) medium (UC2) and small (UC3) size categories (Fig. 2). Basic morphological parameters have been measured to the nearest 0.1 mm on each sagittae by a digital caliper.

Preparation for structural analysis

Samples were embedded in epoxy resin and transverse sections had been prepared using an IsoMet[®] low-speed saw and a Buehler MiniMet grinding-polishing machine. Cut faces were ground using the standard sequence of water-based diamond suspensions (9, 6, 3, 1 μ m; METADI[®]) and alumina (0.05 μ m; Gamma Micropolish[®] II) was used for final polishing. Samples were placed in a distilled water bath and agitated by an ultrasonic cleaning device for 15 min. Sectioning trials were made both perpendicular to (UC1) and parallel with (UC2, UC3) the sagittal plane to see any potential directional difference of structure or mineralogy.

Analytical methods

Cathodoluminescence microscopy

Cathodoluminescence (CL) is an electron-excited phenomenon. Upon bombardment by a high-energy electron beam originating from a cathode, certain materials emit photons in the visible range of the electromagnetic spectrum. Excitation is generated at luminescence centres (activators) in the material, which are crystal lattice defects and chemical impurities. It is generally accepted that Mn^{2+} is the most important activator of CL in carbonate minerals (Marshall 1988; Machel 2000). CL microscopy is a common technique in sedimentary petrology to study the mineralogy, texture and diagenetic history of carbonate rocks (Machel 2000). Biogenic carbonates (Barbin 2000) and fish otoliths (Halden et al. 2004) are also routinely analysed.

Cathodoluminescence examination was performed using a Reliotron cold-cathode equipment mounted on a Nikon E600 microscope. The equipment was operated at 8 to 10 kV accelerating voltage and 0.5 to 1.0 mA current, and a defocused electron beam was used. Photos were taken using a Nikon Coolpix 4500 digital camera using automatic exposure.

Raman microspectroscopy

Raman spectroscopic analysis was done using a HORIBA Jobin Yvon LabRAM HR dispersive, edge-filter based confocal Raman spectrometer (focal length: 800 mm) equipped with an Olympus BXFM microscope. Spectra were taken using the 785 nm emission of a diode laser, a $100 \times (N.A. 0.9)$ objective, a grating with 600 grooves/mm and a pinhole of $100 \,\mu$ m, which acted also as the entrance slit to the spectrometer. Net counting times were between 30 and 60 s. Raman spectroscopy is a routinely used technique to study small-scale mineralogical properties in fish otoliths (e.g. Gauldie et al. 1997; Tzeng et al. 2007).

Results and discussion

Macroscopic and microscopic incremental structures

Darker and lighter bands were clearly observed on each polished section independently of the direction of the sectioning plane (Fig. 2). These coupled bands were described of the recent sciaenid otoliths to indicate an annual increment (annulus) (Arneri et al. 1998; La Mesa et al. 2008; Engin & Seyan 2009). The annual periodicity of annulus deposition was confirmed by the edge analysis in recent sciaenids, indicating that translucent and opaque zones are formed once a year. The opaque zone was laid down in summer, between June and July while the dark/translucent zone was found to be deposited during the rest of the year, starting at the end of summer (September) (La Mesa et al. 2008). The ontogenic age of the specimens can be assigned on the basis of counts of dark/ translucent winter bands (Arneri et al. 1998). The estimated age of UC1 is 17, of UC2 is 7, while of UC3 is 6 years.

The columnar crystal fabric was also perfectly preserved (Fig. 3b) indicating the lack of any diagenetic impact on these fossils.

Estimated somatic parameters

A formalized experimental relationship has been published recently describing ontogenic age vs body length and otolith maximum length vs body weight relationships for brown meagre (*Sciaena umbra* Linnaeus, 1758) (La Mesa et al. 2008), a possible present-day relative to the Pannonian *Umbrina subcirrhosa*. The obtained equations are thought to be robust as they rely on 532 specimens of brown meagre captured over a period of 11 years. Using equations obtained on this modern dataset, somatic parameters of the Pannonian fish specimens can be estimated as 46 cm/1470 g for UC1; 42 cm/1130 g for UC2 and 41 cm/1030 g for UC3.

It is necessary to emphasize that these estimated somatic parameters must be treated with caution. For instances the large difference in the size of the water bodies (i.e. Modern Adriatic Sea and Late Miocene Lake Pannon) and the lack of knowledge about the food web complexity of the paleoenvironment might introduce significant and hardly quantifiable bias.

Preserved mineralogical composition

Otolith cores showed green, yellowish green luminescence. The intensity vanished towards the rim while faint blue luminescence was observed at the outer sectors (Fig. 3a). It is interesting to note that seasonal increments showed different CL characteristics within the annulus. Greenish and weak blue luminescence bands corresponded to the opaque (summer) and dark/translucent (winter) zones, respectively.

CaCO₃ minerals have characteristic CL colours caused by the substitution of Mn^{2+} for Ca²⁺: orange(-red) for calcite and green to yellow for aragonite (Marshall 1988). ~10-20 ppm Mn^{2+} concentration is necessary for visible Mn^{2+} activated luminescence in biogenic calcite and the same or less in aragonite (Barbin 2000). In the case of pure carbonates or carbonates with minor traces of substituting element, a weak blue (so-called intrinsic) CL is detected for both polymorphs.

The observed CL features, consequently, indicate aragonitic mineralogy in the otolith cores but the results are still not conclusive in the outer part. Raman microspectrometric analyses helped to clarify the ambiguity. The Raman spectra



correspond to the aragonite phase (Urmos et al. 1991; Gauldie et al. 1997) proving that the Pannonian otolith undoubtedly contained aragonite crystals at each analysed spot (Fig. 3c). As all the fossil otolith

Fig. 3. Structural evidence for the lack of recrystallization. a - Cathodoluminescence (CL) images. White dots mark the spots for Ramicrospectrometry man (annotation corresponds to the legend in c). Rectangles help to orientate the CL image in the polished section of Fig. 2. b - Radially arranged columnar aragonite crystals in UC3. c - Raman spectra from UC1. Vertical dashed lines indicate the characteristic Raman bands of aragonite.

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material provided only the characteristic Raman bands of aragonite, regardless of the CL colour found in the otolith zone, it is proven that the original aragonitic mineralogy has been preserved also in the parts with weak blue luminescence.

Incidentally, alternating greenish and weak blue CL zones corresponding to opaque and translucent incremental zones of annuli indicate that Mn^{2+} concentration drops below the ~10-20 ppm level in the increment laid down during the winter season.

Conclusions

Macroscopic and microscopic observations of the three studied Pannonian (Late Miocene) sciaenid otoliths offered clear evidence for the preservation of the genuine structural characteristics. Ontogenetic ages were assigned for the three specimens as 16, 7 and 6 years, respectively, by counting the annuli of sagittae. Corresponding somatic parameters of the fish specimens can be estimated as 46 cm/1470 g; 42 cm/1130 g and 41 cm/1030 g using experimental physiological relationships of the brown meager (*Sciaenia umbra* Linnaeus, 1758) a modern relative of the studied Pannonian species.

Results from CL and Raman microspectrometric analyses proved the preservation of the original aragonitic mineralogy. The excellent preservation of these fossils invites further microchemical studies to reconstruct the past physicochemical environment. As earlier geochemical/paleoecological studies concentrated exclusively on benthic organisms, these otoliths might provide the first geochemical record from Pannonian nektonic organisms related directly to the open water marine/lacustrine conditions free from any bias due to the benthic environment.

Acknowledgments: The European Union and the European Social Fund have provided financial support to the Project under the Grant agreement No. TÁMOP 4.2.1./B-09/KMR-2010-0003. The research has been supported by Baross Gábor Fund REG-KM-09-1-2009-0044 and OTKA K67583 grants. The authors thank Ágnes Takács for her help with sample preparation. Rostislav Brzobohatý and Norman Halden are acknowledged for their constructive reviews. This is Budapest Tree-Ring Laboratory contribution No. 20.

References

- Arneri E., Colella S. & Giannetti G. 1998: A method for the age determination of two Mediterranean sciaenids: *Sciaena umbra* (Linnaeus, 1758) and *Umbrina cirrosa* (Linnaeus, 1758). *Rapp. Comm. Int. Mer Médit.* 35, 366–367.
- Barbin V. 2000: Cathodoluminescence of carbonate shells: biochemical vs. diagenetic process. In: Pagel M., Barbin V., Blanc P. & Ohnenstetter D. (Eds.): Cathodoluminescence in geosciences. *Springer*, Berlin/Heidelberg, 303-329.
- Bosnakoff M. 2008: Late Miocene (Pannonian) sciaenid fish otoliths from Hungary — preliminary studies. *Hantkeniana* 6, 219-228.
- Bosnakoff M. & Katona L. (in print): Fish otolith assemblage from the Late Miocene deposits of Lake Pannon (Doba, NW Hungary). *Central European Geol.*
- Brzobohatý R. & Pana I. 1985: Die Fischfauna des Pannonien. In: Papp

A., Jámbor Á. & Steininger F.F. (Eds.): Chronostratigraphie und Neostratotypen, Miozän der Zentralen Paratethys 7, Pannonien. *Akadémiai Kiadó*, Budapest, 426-439.

- Carlström D. 1963: A crystallographic study of vertebrate otoliths. *Biological Bull.* 125, 441-463.
- Carpenter S.J., Erickson J.M. & Holland F.D. 2003: Migration of a Cretaceous fish. *Nature* 423, 70-74.
- Cziczer I., Magyar I., Pipík R., Böhme M., Corić S., Bakrač K., Sütő-Szentai M., Lantos M., Babinszki E. & Müller P. 2009: Life in the sublittoral zone of long-lived Lake Pannon: paleontological analysis of the Upper Miocene Szák Formation, Hungary. *Int. J. Earth Sci.* 98, 7, 1741-1766.
- Engin S. & Seyan K. 2009: Age, growth, sexual maturity and food composition of *Sciaena umbra* in the south-eastern Black Sea, Turkey. J. Applied Ichthyology 25, 96–99.
- Gauldie R.W., Sharma S.K. & Volk E. 1997: Micro-Raman spectral study of vaterite and aragonite otoliths of the coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol. A 118, 753–757.
- Geary D.H., Rich J.A., Valley J.W. & Baker K. 1989: Isotopic evidence for salinity changes in the Late Miocene Pannonian basin: effects on the evolutionary radiation of melanopsid gastropods. *Geology* 17, 981–985.
- Geary D.H., Magyar I. & Müller P. 2000: Ancient Lake Pannon and its endemic molluscan fauna (Central Europe; Mio-Pliocene). Advances in Ecological Res. 31, 463-482.
- Halden N.M., Marthers K., Babaluk J.A. & Mejia S.R. 2004: Cathodoluminescence microscopy: A useful tool for assessing incremental chemical variation in otoliths. *Environmental Biology of Fishes* 71, 53-61.
- Kázmér M. 1990: Birth, life and death of the Pannonian Lake. Palaeogeogr. Palaeoclimatol. Palaeoecol. 79, 171-188.
- Kuhlemann J., Frisch W., Székely B., Dunkl I. & Kázmér M. 2002: Post-collisional sediment budget history of the Alps: Tectonic versus climatic control. *Int. J. Earth Sci.* 91, 5, 818–837.
- La Mesa M., Colella S., Giannetti G. & Arneri E. 2008: Age and growth of brown meagre *Sciaena umbra* (Sciaenidae) in the Adriatic Sea. *Aquatic and Living Res.* 21, 153–161. DOI: 10.1051/alr.2008029
- Leeder K. 2007: Foreland of the Romanian Carpathians. Controls on late orogenic sedimentary basin evolution and Paratethys palaeogeography. *PhD Thesis*, *Vrije Universiteit*, Amsterdam, 1–182.
- Machel H.G. 2000: Application of cathodoluminescence to carbonate diagenesis. In: Pagel M., Barbin V., Blanc P. & Ohnenstetter D. (Eds.): Cathodoluminescence in geosciences. *Springer*, Berlin/ Heidelberg, 271-301.
- Magyar I., Lantos M., Ujszászi K. & Kordos L. 2007: Magnetostratigraphic, seismic and biostratigraphic correlations of the Upper Miocene sediments in the northwestern Pannonian Basin System. *Geol. Carpathica* 58, 277-290.
- Marshall D.J. 1988: Cathodoluminescence of geological materials. Unwin-Hyman, Boston, MA, 1-146.
- Mátyás J., Burns S.J., Müller P. & Magyar I. 1996: What can stable isotopes say about salinity? An example from the Late Miocene Pannonian Lake. *Palaios* 11, 31-39.
- Müller P., Geary D.H. & Magyar I. 1999: The endemic molluscs of the Late Miocene Lake Pannon: their origin, evolution, and familylevel taxonomy. *Lethaia* 32, 47-60.
- Tóth E., Görög Á., Lecuyer C., Moissette P., Balter V. & Monostori M. 2010: Palaeoenvironmental reconstruction of the Sarmatian (Middle Miocene) Central Paratethys based on palaeontological and geochemical analyses of foraminifera, ostracods, gastropods and rodents. *Geol. Mag.* 147, 2, 299-314.
- Tzeng W.N., Chang C.W., Wang C.H., Shiao J.C., Iizuka Y., Yang Y.J., You C.F. & Ložys L. 2007: Misidentification of the migratory history of anguillid eels by Sr/Ca ratio of vaterite otoliths. *Marine Ecology Progress Series* 348, 285–295.
- Urmos J., Sharma S.K. & Mackenzie F.T. 1991: Characterization of some biogenic carbonates with Raman spectroscopy. *Amer. Mineralogist* 76, 641–646.
- Woydack A. & Morales-Nin B. 2001: Growth patterns and biological information in fossil fish otoliths. *Paleobiology* 27, 369–378.