Title: Geochemical methods of inference the termoregulatory strategies in Middle Triassic marine reptiles-a pilot study

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Citation style: Surmik Dawid, Pelc Andrzej. (2012). Geochemical methods of inference the termoregulatory strategies in Middle Triassic marine reptiles-a pilot study. "Contemporary Trends in Geoscience" (Vol. 1 (2012), s. 87-91), doi 10.2478/ctg-2012-0013

DOI: 10.2478/ctg-2012-0013

GEOCHEMICAL METHODS OF INFERENCE THE THERMOREGULATORY STRATEGIES IN MIDDLE TRIASSIC MARINE REPTILES – A PILOT STUDY

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Abstract. The oxygen stable isotopes investigation to elucidate thermoregulatory strategies in Middle Triassic basal sauropterygians is currently ongoing at University of Silesia and University of Maria Curie-Skłodowska. The results of similar studies on Late Mesozoic marine reptiles indicate that some of fully aquatic reptiles like plesiosaurs or ichthyosaurs could be warm-blooded animals.

Our investigation is an important part of the aim of the research project "The Marine and Terrestrial reptiles in the Middle Triassic environmental background of Southern Poland" to solve the thermoregulation issue in basal marine reptiles and show how, and when did homoiothermy evolve in Sauropterygia..

Homeothermy and gigantothermy were important physiological adaptations which allowed sauropterygian ancestors to leave the shores and conquer the open seas and oceans.

Keywords:. Sauropterygia, Middle Triassic, homeothermy, poikilothermy, oxygen stable isotope geochemistry.

Introduction

Chemical and isotope analyses of skeletal remains are currently widely used in forensic science, as well as archeology and paleobiology to determine the primary origin of the investigated person or specimen.

A detailed knowledge of metabolic processes, body temperatures, as well as growth rates in extinct vertebrates is very difficult to obtain. Fortunately, stable isotope ratios of bioapatite may be influenced by biological processes in identifiable ways that provide paleobiological insight (Fricke, 2007).

The stable isotope investigation may provide an answer on the nature of environmental conditions, differences in the behavior, dietary choices, and preferred habitats of extinct animals based on various conditions, like chemical compositions of water and diet, which is characteristic to specific geographic regions.

The tissues of living animals contain carbon, oxygen, nitrogen, and other elements which are sourced from the food, water, and air that animals ingest and respire respectively

Dietary components have a strong influence on chemical composition of animal bones (Deniro & Epstein, 1981).

According to the rule "*you are what you eat*", the herbivorous animals (plant eaters) have carbon-rich bones, however the carnivores (meat eaters), are nitrogen-rich. Omnivorous animals, like humans or pigs have a mixed (plant and meat) diet, so also the have a mixed carbon-nitrogen isotope ratios in their bones.

The carbon stable isotopes ratios (C^{13}/C^{12}) also can answer the question on whether the food components of herbivorous animals were C3 (Calvin pathway plants), or C4 (Hatch-Slack pathway) plants. These different metabolic pathways in plants are characterized by different isotopic discrimination and variability (Fricke, 2007). Therefore this method can indirectly show the changes in floristic world.

As well as the diet, the stable isotope analysis can also give an answer on some physiological details, for example thermoregulation.

The studies on the paleophysiology of fossil vertebrates hasbecome the subject of interestin recent years.

These studies consist of compiling data from a number of complementary methods such as physiology (the study of modern animals, in accordance to the principle of uniformitarism) and isotope geochemistry.

Of particular interest is the problem of thermoregulations of fossil reptiles, which is a source of lively debate by the dinosaur researchers (Reid, 1997, Ruben et al., 1996; Burness et al., 2001; Eagle et. al, 2011). Determination of oxygen stable isotope ratios in tooth enamel have been used to solve the thermal metabolism strategy in the obligatory marine reptiles from Jurassic and Cretaceous (Bernard et al., 2010; Motani, 2010).

The results of these investigations indicate that Late Mesozoic animals like plesiosaurs and ichthyosaurs could be homoethermic (endothermal, warm-blooded). In the latter case, the control sample used for isotope analysis was that of fish teeth, which was intended to assess the possible impact of environmental conditions on the isotope signature (Bernard et al., 2010).

Early marine reptiles, like nothosaurs and placodonts have probably been semi-aquatic in mode of life so a poikilothermic (ectothermal, cold-blooded) metabolism is most likely. However large body sizes may indicate gigantothermy, a situation in which when large ectothermic animals are more easily able to maintain a constant, relatively high body temperature (Eagle et al., 2011).

Material and methods

Stable isotope fractionation. Isotopes of elements are differentiated from each other by the number of neutrons in the atomic nucleus and these differences result in a fractionation if heavy and light isotopes during physical, chemical, and biological processes. Differences in the relative abundance of heavy to light isotopes (so called isotope ratio) in various materials can be prominent, particularly when environmental fluctuations or physiological reactions are ongoing (Fricke, 2007).

Samples. Bioapatite is a major inorganic component of different skeletal elements, including teeth and bones. Teeth consist of two main kinds of materials: an enamel and dentine. The enamel forms a hard shell around the softer dentine core.

Generally, teeth grow incrementally for a limited period early in the life of an animal, so isotopic ratio is recorded in teeth, especially in enamel shell.

Bone can be remodeled over the entire animal’s life, so this is the worst information medium and stable isotope ratios in its bioapatite may reflect conditions prior to death (Fricke, 2007).

The examined samples are teeth (enamel or dentine and enamel) of nothosaurs (several dozen teeth) and placodonts (enamel from five teeth). Due to the necessary sample mass (at least 50mg) in some cases we would use whole tooth specimens. In mentioned cases obtained data will be averaged datasets from enamel and dentine.

The samples came from earliest Anisian – uppermost Ladynian carbonate deposits from Upper Silesia.

Oxygen stable isotopes analyses were performed by Laboratory of Mass Spectrometry at Institute of Physics, Maria Skłodowska-Curie University in Lublin, Poland.

Investigating of oxygen stable isotope ratios was planned as an important aspect of the research project "The Marine and Terrestrial reptiles in the Middle Triassic environmental background on Southern Poland" to solve the thermoregulatory strategies in primitive marine reptiles.

Experimental. For the investigation of the oxygen isotopic composition of the tooth phosphate the Stephan (2000) protocol was used. In this procedure the tooth phosphates are converted to silver orthophosphate (Ag_3PO_4). For mass spectrometric analysis at least 50mg of the material is needed. At the beginning the material was powdered and then the organic and humic substances were removed from the sample by treatment with NaOCl and NaOH. Residues were then dissolved in the HF solution. After the separation of the insoluble part of the sample, the remaining solution with the phosphate fraction was neutralized with KOH solution. After the solution was neutralizes the buffered amine solution ($AgNO_3$, NH_4NO_3, NH_4OH and H_2O) was added. The solution was then gradually warmed up to 70°C. This temperature was held for 3 hours, and then the solution wascooled down slowly to the room temperature. During this process the Ag_3PO_4 precipitates. The obtained silver orthophosphate was then washed several times with distilled water and dried.

By Having the Ag_3PO_4 we can analyze the oxygen isotope composition of phosphates from the tooth material. The extraction of the oxygen from the Ag_3PO_4 was done using a newly developed graphite reduction method (Pelc & Halas, 2010). Below is a brief description of the oxygen extraction procedure.

At the beginning of the oxygen extraction, both reagents obtained from the tooth Ag_3PO_4 and graphite (Sigma Aldrich, Poznań, Poland) were roasted in vacuum at 500ºC and 900ºC, respectively. Then the Ag_3PO_4 was mixed thoroughly in agate mortar with graphite in 2:1 ratio. The quantity of about 40 mg of this mixture was loaded into a platinum boat. The boat with the sample was then installed into glass reaction chamber. In the chamber the boat with the sample is resistively heated. The boat temperature is controlled by the voltage of the power supplied to the ends of the boat. To avoid a sample spillage, the reaction chamber was slowly evacuated to attain a pressure below 10^{-3} mbar. Then degassing of the sample was done at 200ºC over about 20 min in order to remove any volatile impurities. After degassing the boat temperature was raised gradually to 750°C. At these conditions the reduction reaction of silver orthophosphate with graphite proceeds over about 30 min.

In the reduction reaction the CO_2 and CO gases are produced, with efficiency depending on the boat temperature. In as much as CO_2 gas is more convenient for the mass spectrometric analysis than CO, the CO fraction was converted to CO_2 by the glow discharge between two Pt electrodes installed in the conversion chamber. The CO_2 gas produced is cryogenically collected on the inner walls of the conversion chamber immersed in liquid nitrogen. As the CO gas may be trapped in solid CO_2, the conversion chamber was heated by a hot gun to sublimate the condensed gases. Then CO_2 was frozen again, and the released CO was converted to CO_2. This procedure was repeated twice. The CO_2 collected was then cryogenically transferred to the sample ampoule.

After the CO_2 collection the sample ampoule was connected to the inlet system of the isotope ratio mass spectrometer with triple collector for the determination of the oxygen isotope ratio.

Since the oxygen extraction in the graphite reduction of silver orthophosphate is incomplete, the obtained isotope composition has to be corrected by +0.7‰. This correction was precisely determined by the analysis of standards determined with the fluorination method that assures the complete oxygen extraction from the Ag_3PO_4 (Halas et al., 2011).

Discussion

Body sizes and thermoregulatory strategies. It seems that the animals living in near-shore and marginal parts of epicontinental basin should be cold-blooded.

The absence of the hypothalamic body temperature-control mechanism was probably the one of limiting factors of the open seas expansion. The extant marine iguanas (*Amblyrhynchus cristatus*) and monitor lizards (Varanidae) inhabit similar environmental niches, and never gain the open waters.

During the visit in the Museum für Naturkunde in Berlin, Germany, one of us (DS) had opportunities to investigate the bone material from Rüdersdorf and Jena outcrops in Eastern Germany. The biggest part of the material consists of very huge limb bones, indicating that those animals can grow to sizes exceeding 6 meters.

Is this possible, that cold-blooded animals could grow so large? The biggest monitor lizard, *Varanus komodoensis* is the largest living species of lizard (Burness et al., 2001), and grows to a maximum length of 3.5 meters, a typical size of large nothosaurs.

Gigantothermy, known in late Mesozoic marine reptiles such as mosasaurs and ichthyosaurs as well as extant leatherback turtles, is a phenomenon of maintaining the high body temperature due to the large sizes (Bernard et al., 2010).

Gigantothermy seems to be possible in respect of the largest nothosaurs, pistosaurs, and particularly of the large fully-aquatic ichthyosaurs like *Shastasaurus* or 10-meter *Cymbospondylus*, known *inter alia* from Middle Triassic European Basin.

On the other hand, the aforementioned "Komodo dragon" is also an example of a phenomenon, called "insular gigantism" in which the size of animals isolated on an island, where there are no natural enemies, increases dramatically. In Triassic epicontinental seas nothosaurs also had no enemies, in particular in the marginal parts of seas (Surmik, 2010).

Environmental settings and control samples. It is significant, that environmental factors have an influence on isotope fractionation. Some factors like sea bathymetry, climate, original water chemistry (e.g. salinity) and secondary, diagenetic chemical alterations associated with the thermal maturity of rocks can distort the primary (physiological) isotope signature (Lècuyer, 2004).

Nevertheless, the fossil material from Silesia is characterized by excellent quality: a perfect state of tooth enamel preservation and large abundance of aforementioned material. Additionally, a level 1 result on the conodont Color Alteration Index (the CAI) (Repetski & Narkiewicz, 1996), indicates a lower thermal maturity of fossil material, and an abundance of control samples, for example fish teeth, and conodonts. Conodont phosphates are the best medium for geochemical information, and in Middle Triassic sedimentary rocks they are relatively common fossils.

For comparative purposes we would like to use also teeth of the extant Galapagos marine iguana (*Amblyrhynchus cristatus* Bell 1825), as an ecological proxy of extinct nothosaurs and placodonts. This iguanid lizard lives in similar near-shore environments and has known thermoregulatory strategy.

Seasonality. In case of ectothermic animals varying environmental temperatures also could be mirrored by the body temperature. Fish teeth and conodont material reflect the environmental temperatures of sea water.

An intra-tooth sampling of a single fish tooth provides information for only one season in the life of a single individual. Studies of multiple teeth and/or conodonts from more than one individual will more likely provide a representative sample of the environmental conditions experienced by a population of animals (Fricke, 2007).

It is important to characterizing seasonal variations in tooth enamel isotope ratios involves the analysis of many samples of tooth enamel from the same taxon and from the same sedimentary beds, that shows – as far as possible – slight time-averaging and similar bathymetry.

Physiology of homeothermic thermoregulatory. In case of human, normal body temperature is approx. 36.6 degree Celsius, because the highest enzyme activity is located just in the range 36-37 degree Celsius.

Most homeothermic animals have similar body temperature. An estimate of body temperatures in case of Late Mesozoic marine reptiles (Bernard, et al. 2010), rangeing from 35 to 39 degrees Celsius, suggest high metabolic rates required for predation and fast swimming over large distances offshore. In the case of sauropods (gigantothermy), body temperatures “read” from isotope ratios are 37,7-38,2 degree Celsius (Eagle, et al., 2011). When isotopic signatures from tooth material indicate temperatures significantly more than 30 degree Celsius, this implies the result is a result of body temperatures, not environmental impact. Nowadays, the highest water surface temperature exceeds 30 degree Celsius in low-latitude Persian Gulf in the territory of United Arab Emirates, Bahrain, Qatar and Oman (Delphi & Mosaddad, 2010).

Complementary data. Alternatively, the geochemical and structural analyses of fossilized excrements (coprolites) is also planned.

The elemental and phase composition of the sample, as well as the degree of food remnants degradation is helpful to understand the metabolic rate and indirectly draw conclusions about the way of thermoregulation.

The microstructural, XRD and EDS analyses will be performed in Faculty of Earth Science, University of Silesia, Sosnowiec, Poland.

Conclusions

Using the modern investigating methods is extremely important to complement the current state of knowledge about biology of Middle Triassic marine reptiles. The early evolution of these reptile groups that flourished in Mesozoic times, including the elucidation of the origin of some particular marine reptile subgroups as well as physiological adaptations to conquest the Mesozoic seas.

Investigating material from the same sauropterygian taxa for a significant period of time (earliest Anisian – uppermost Ladynian) may give an answer on the question about how and when homeothermy evolved in Sauropterygia.

Acknowledgments

An opportunity to investigate the fossil materials deposited in Museum fur Naturkunde in Berlin, Germany has been financed under the SYNTHESYS research project, DE-TAF-1746 "*Taxonomical diversification of the Middle Triassic marine reptiles from European Basin*". The funds to SYNTHESYS are made available through the European Commission.

The teeth of the marine iguana were made available courtesy of Dr. Timothy Bromage, Hard Tissue Research Unit, Department of Biomaterials and Biomimetics, New York University.

This research project is supported by the National Science Centre grant no. DEC-2011/01/N/ST10/06989 "*The Marine and Terrestrial reptiles in the Middle Triassic environmental background on Southern Poland*".

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Abstrakt

Badania nad paleofizjologią kopalnych kręgowców ostatnimi laty stały się niezwykle modne. Polegają one na kompilacji danych uzyskanych wieloma komplementarnymi metodami z zakresu fizjologii (badania współczesnych form, zgodnie z zasadą aktualizmu) i geochemii izotopowej. Szczególnie interesujące stały się kwestie gospodarki termicznej u gadów kopalnych, które silnie dyskutowane są w kręgach badaczy dinozaurów (Reid, 1997; Ruben i in., 1996).

Badania na izotopach stabilnych tlenu szkliwa zębowego przeprowadzone na obligatoryjnie morskich gadach okresu jurajskiego i kredowego (Bernard i in., 2010; zob. także Motani, 2010) wskazują, że ichtiozaury i plezjozaury późniejszego mezozoiku mogły być zwierzętami stałocieplnymi. Brak obecnie jednoznacznych danych dotyczących gospodarki termicznej bazalnych przedstawicieli gadów morskich z triasu, choć przyjmuje się, że te zamieszkujące nadbrzeżne i marginalne strefy mórz zwierzęta były gadami zmiennocieplnymi (pojkilotermicznymi), podobnie jak współczesny legwan morski, czy też smok z Komodo.

Czy przejście z pojkilo- do homojotermii było jedną z adaptacji umożliwiających mezozoicznym gadom morskim ekspansję na otwarte morza? Na pytanie w jaki sposób ewoluowała homojotermia u gadów morskich spróbuje odpowiedzieć projekt badawczy pt. "Gady morskie i lądowe na tle środowiska triasu środkowego południowej Polski", realizowany na Uniwersytecie Śląskim.

Słowa kluczowe: Sauropterygia, gady morskie, środkowy trias, homojotermia, pojkilotermia, geochemia izotopowa, izotopy stabilne tlenu.