Reports

Isotopic Evidence on the Early Life History of Nautilus pompilius (Linné)

Abstract. *The ratios of oxygen-18 to oxygen-16 and of carbon-13 to carbon-12 in the early shell and septa of two* Nautilus *specimens are interpreted for the early ontogeny of the animals. Changes in the carbon isotope content are tentatively correlated with the end of the embryonic period and with environmental changes. Variations in the oxygen isotope content are in part ascribed to migrations from warm to cooler water after a certain stage of development. The size of the body chamber of the young animals can be determined by comparison of the isotope contents in outer shell and septa.*

The investigations on fossil cephalopods of Erben (*1*), Ristedt (*2*), Schindewolf (*3*), and Shimanskiy and Zhuravleva (*4*) have indicated the importance of the early ontogenetic stages in the elucidation of phylogenetic relationships. A detailed knowledge of the embryology of the sole surviving member of the ectocochliate cephalopods, that is *Nautilus*, should therefore be important for the interpretation of fossil material. The existing ideas concerning early ontogeny of *Nautilus* are based mainly on morphological criteria of the early shell and on the comparison with somewhat better known, but rather distant, relatives such as *Sepia*. Our research shows that there are also some morphological features which indicate an interpretation of the early life history of *Nautilus* different from that now understood. Up to now no one has been successful in breeding *Nautilus*, and only very little is known about the early ontogeny by direct observation.

We have tried to solve some of the problems of early development by means of carbon and oxygen isotope investigations. A successive sequence of septa and outer-shell increments between the septa, prepared free from the shell material of the following whorl, from the protoconch forward from two *Nautilus pompilius* specimens was investigated for the ratios, O^{18}/O^{16} and C^{13}/C^{12}. These subsamples comprise the complete carbonaceous layer sequence in outer shell and in the septa, and no separation in different shell layers was undertaken. Organic material attached to the shell carbonate was removed by a 5.2 percent sodium hypochlorite solution. Details of the sample treatment are given by Keith *et al.* (*5*). The preparation of the gas samples prior to analysis with a modified Nier-type mass spectrometer was essentially the same as that described by Epstein *et al.* (*6*). Data are recorded in δ values, as parts per thousand difference of O^{18} and C^{13} content, relative to the Chicago PDB_I standard CO_2. The reproducibility of the data, including deviations due to sample preparation, is better than or equal to ±0.2 per mill. The *Nautilus* specimens were obtained from the area of the Philippine Islands, but the exact location is not known.

The oxygen isotope content (Fig. 1) in the shell increments of both *Nautilus* specimens varies between $\delta O^{18} = -3.0$ and $+0.9$ per mil. The O^{18} content in the $CaCO_3$ of the septa ranges between -4.7 and $+1.0$ per mil. The isotope variations are very similar in both specimens. Nearly all of these O^{18}/O^{16} ratios probably can be accounted for by the well-established fact that the oxygen isotope ratio in carbonate depends on the water temperature at the time of formation of carbonate. The temperature data in Fig. 1 corresponding to the δO^{18} values are derived from the equation given by Epstein *et al.* (*6*). An oxygen isotope content of 0.0 per mil relative to PDB_I of the sea water, in which the carbonate was deposited (and which goes into the equation), was compiled from the isotope values (*7*) of the sea water at the Palau Islands and at Tumon Bay in Guam. The actual water temperature in which the shell carbonate was formed should fall within ±2°C of the calculated values.

From this follows that specimen I (Fig. 1*A*) was living in relatively warm water of about 24°C up to the time of deposition of the 12th shell increment. According to Willey (*8*) the eggs of *Nautilus* become attached when they are deposited, and therefore the very early development apparently takes place in this temperature region. A comparison of the temperatures with the temperature-depth conditions of the sea water at Palau (Motoda, in Wells, *9*) indicates a depth of water of about 80 to 110 m. After a certain age, the animal then apparently wandered away rather suddenly into much cooler and probably deeper water of about 17° to nearly 13°C. In this region the outer-shell increments starting from the 13th were thus deposited. The depth of water in Palau corresponding to these temperatures would be about 200 to 300 m. At the time of migration the animal must already have left the egg. Specimen II (Fig. 1*A*) showed similar variations of the oxygen and carbon isotope ratios, apart from exhibiting a rather low O^{18} content in the carbonate of increment 10 which would correspond to a temperature of about 30°C. The depth conditions derived in this manner agree quite well with the mode of life of *Nautilus* as observed in nature (*10*).

A comparison of the relative oxygen isotope variations in the carbonate of the septa (Fig. 1*B*) with the ones in the outer-shell increments makes further deductions possible. The probability is high that the sudden change in the O^{18} content between septa 7 and 8 is equivalent to the change in the outer-shell increments discussed above from 12 to 13, and from 10 to 12, in specimen II. In other words, septum 7 was formed at the same time and temperature as shell increment 12 in specimen I, and septum 8 was incorporated in cooler water at the time when shell increment 13 was deposited. One thus obtains a measure for the size of the body chamber of the young animal and indirectly also of the soft parts themselves. In specimen I, the body occupies the volume contained in five shell increments; in specimen II in four shell increments. Even though the relative isotope changes are very similar in both septa and outer shell, the absolute isotope contents do not always coincide exactly. This probably is due to the fact that in the septa practically the only material available for analysis is the carbonate from the nacreous layer. In the outer shell not only nacreous layer but also the spherulitic- and semiprismatic layers (*11*) contribute to each sample. Measurements by Emiliani (*12*) of different shell layers of *Nautilus* indicate that inner and outer layers differ somewhat in their relative isotope contents and this is probably due to the different times of lime deposition.

It is very remarkable that septa 7 and 8, which are usually closer packed are the septa that were apparently deposited directly before and after the change in environment. This phenomenon of closer-packed septa is often regarded as indicating the end of the embryonic phase. Even though the exact mechanism is not fully understood, it appears that this crowding of the septa is rather an indicator and a result of a temporary decrease in the rate of the physiological processes on the transition from warm to cooler water temperatures. Finally, it should be noted that the first three septa of specimen I, and probably the first two in specimen II, exhibit an extremely low O^{18} content relative to the other samples. With respect to the temperature dependence of the O^{18}/O^{16} ratio this would correspond to temperatures of about 40°C, which seems unlikely. This phenomenon could perhaps reasonably be accounted for with the assumption that these septa were incorporated into the shell while still within the egg. The "light" isotope content would then quite reasonably be the result of an isotope fractionation due to diffusion processes within the membranes of the egg capsule. According to Willey (*8*) there are two membranes, an inner and an outer.

The carbon isotope ratios in the $CaCO_3$ of the outer-shell increments and the septa have a spread from $\delta C^{13} = -1.4$ up to $+1.5$ per mil. As with the oxygen isotope results, the C^{13}/C^{12} ratios in the carbonate of both specimens coincide closely (Fig. 2). It is still not quite understood how the carbon isotope composition in the shell carbonate is brought about. Investigations by Keith *et al.* (*5*) on recent mollusc shells indicate that the metabolism and in particular probably the food play a major role in producing the C^{13}/C^{12} variations in the shell. Starting from this hypothesis one could expect a different C^{13} content in the shell increments which were deposited within the egg with yolk as principal food source (as well as a different rate of the physiological processes) than in the carbonate which was secreted after the animal had hatched and begun an active search for food. Similarly one should expect a noticeable change in the carbon isotope composition when there are changes in the rate of physiological processes in connection with changes in environment. As a matter of fact, the first distinct change in the carbon isotope content occurs in the shell region which apparently was deposited in warm water. It is noteworthy that this change in the isotope content occurs in both specimens in the same ontogenetic stage and that is between shell increments 5 and 6. These data seem to suggest that at this stage of development the animals hatched from the eggs.

Later on in the ontogenetic development the "normal" carbon isotope composition is interrupted again by a relatively sudden second change of the isotope content into the C^{13}-deficient region (Fig. 2*A*). This low C^{13} content within the isotope sequence of the outer shell coincides directly with

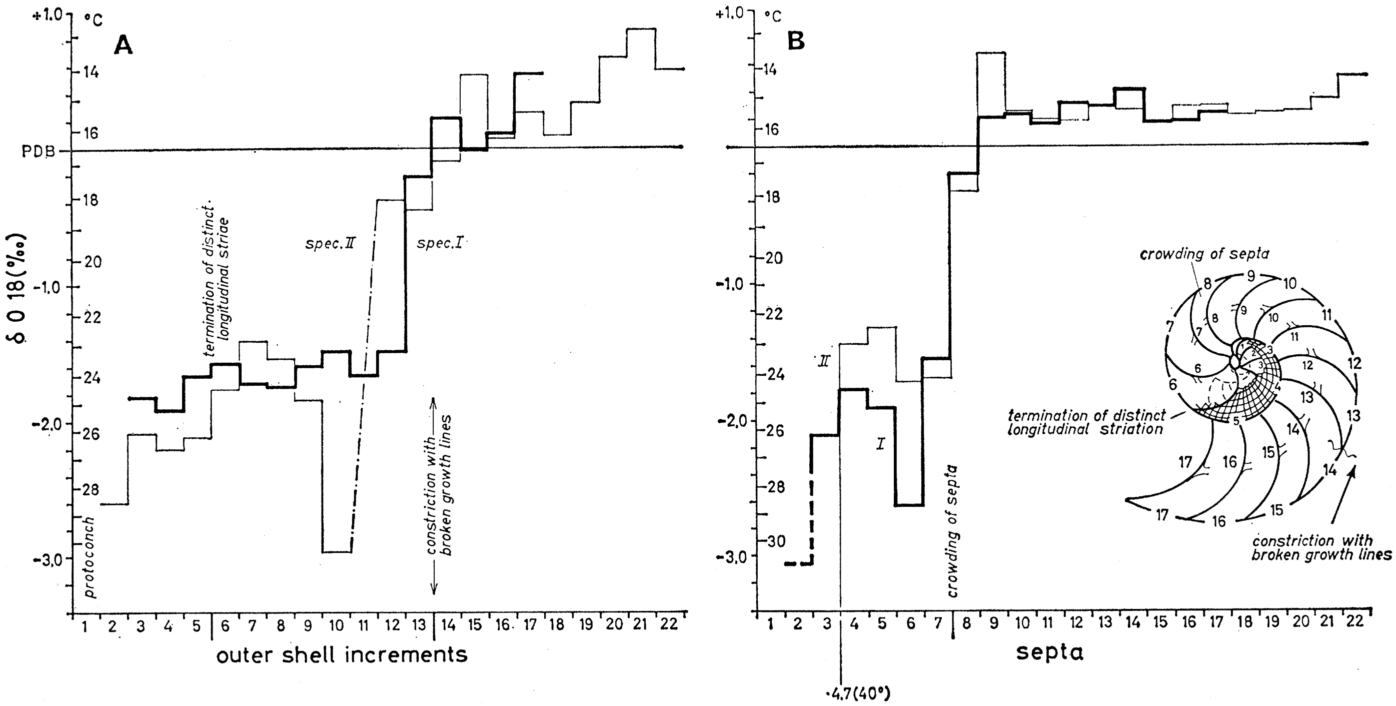

Fig. 1. Oxygen isotopic composition and temperature equivalents in outer-shell increments (*A*) and septa (*B*) from two *Nautilus* specimens.

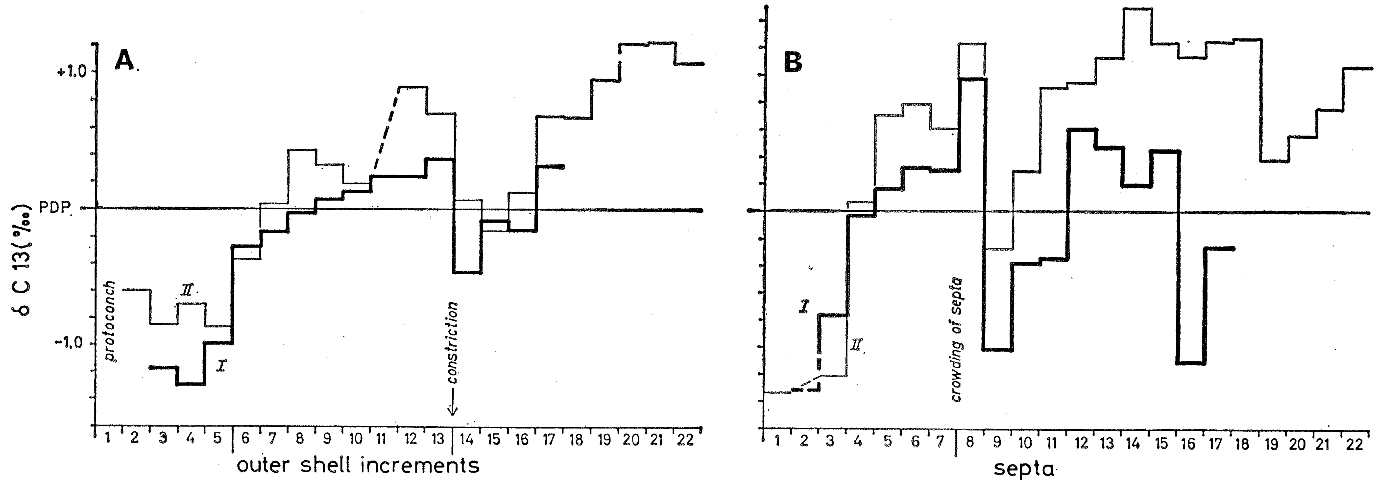

Fig. 2. Carbon isotopic composition in outer-shell increments (*A*) and septa (*B*) from two *Nautilus* specimens.

a line of discontinuous growth, marked by a shallow groove which is best developed on the flanks and at the umbilical shoulders of the shell. This constriction, which morphologically is in many cases accompanied by markedly broken growth lines and former apertural margins that have been injured and thereafter repaired, has been interpreted, for example, by Stenzel (*13*) and by Naef (*14*) as marking the end of the embryonic period. However, the change in the O^{18} content up to two shell increments before the constriction; the change in the C^{13} content between shell increments 5 and 6; its coincidence with certain morphological features, such as the end of the distinct cancellate sculpture or the presence of a curvature maximum of the shell in this region, and the dimensions of egg relative to early shell, as well as injuries of outer shell in very early stages (*15*), appear to indicate a different interpretation. The data suggest that the constriction does not result from the transition from the embryonic phase to the unconfined postembryonic period. This transition appears to take place earlier during the deposition of shell increments 5 or 6. Since constriction and the second change in the carbon isotope content are found only one shell increment after the time of migration into cooler surroundings, it seems possible that these features too are the result of the marked change in the physiological activity or living conditions, or both, with perhaps a different food supply due to the change in environment. Minima of low C^{13} content in the isotope sequence, such as are present in this case, apparently can occur again in later growth stages. This becomes evident from the C^{13} variations in the septa (Fig. 2*B*). As with the oxygen isotope variations, the changes in the carbon isotope content in the shell increments can be compared with the variations in isotope contents of the septa. The sudden change in the isotope content in the shell samples from 13 to 14 thus very likely is equivalent to the isotope change from septum 8 to 9. Apart from the oxygen isotopes, a similar size of five shell increments is ascertained for the body chamber of the young *Nautilus*. A second C^{13} variation between septa 15 and 16 in specimen I, and between 18 and 19 in specimen II, respectively, is therefore beyond the sequence of the outer-shell increments analyzed. A further similarity to the deductions from the oxygen isotopes is provided by the rather low C^{13} content of the first two or three septa. It is more or less equivalent to the carbon isotope composition of the outer-shell carbonate which most likely is deposited during the embryonic period. It seems probable also from the carbon isotope composition that these septa were formed before hatching from the egg.

The isotope ratios thus shed a new and different light upon the hitherto prevailing conception of the early life history of *Nautilus*. Even though only two specimens have been investigated so far with respect to their isotope content, we conclude that these results are basically valid for other specimens on the grounds of the close agreement (i) between both specimens, (ii) between the results from oxygen as well as carbon isotope measurements, and (iii) between isotope results and morphological features. The definite solution of the problems involved, however, will be possible only when the early ontogeny of living *Nautilus* becomes known either by observation in nature or when we succeed in breeding individuals.

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