

# INTRA- AND INTERTAXON STABLE O AND C ISOTOPE VARIABILITY OF FOSSIL FISH OTOLITHS: AN EARLY EOCENE TEST CASE

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## KEYWORDS

early Eocene climatic optimum  
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 stable isotope  
 fish otolith

## ABSTRACT

Knowledge of basic data variability is essential for the interpretation of any proxy-based paleotemperature record. To evaluate this for  $\delta^{18}\text{O}$  stable isotope paleothermometry based on early Paleogene fish otoliths from marginal marine environments, an intra- and interspecific stable O and C isotope study was performed at a single locality in the southern North Sea Basin (Ampe Quarry, Egem, Belgium), where shallow marine sands and silts are exposed. The age of the deposits is early late Ypresian (ca. 50.9 Ma) and falls within the early Eocene climatic optimum (EECO) interval. In each of four fossiliferous levels sampled, the same three otolith species were analyzed (*Platycephalus janeti*, *Paraconger papointi* and "genus Neobythitiorum" *subregularis*). Intrataxon stable isotope spread amounts on average 2.50-3.00‰ for all taxa and is present in all levels. This implies that each sample level comprises substantial variability, which can be attributed to a combination of temporal and taphonomic effects. More importantly, intertaxon offsets of 4.60‰ in  $\delta^{13}\text{C}$  and 2.20‰ in  $\delta^{18}\text{O}$  between the mean values of the three otolith species are found, with "N." *subregularis* representing more positive values relative to the other species. We hypothesize that freshwater influence of coastal waters is the most likely cause for these discrepancies. Similar analyses on two coastal bivalve species (*Venericardia sulcata* and *Callista laevigata*) corroborate this hypothesis. Accordingly,  $\delta^{18}\text{O}$  values measured on "N." *subregularis* otoliths probably represent a more open oceanic signal, and therefore seem well-suited for  $\delta^{18}\text{O}$  stable isotope paleothermometry. This study highlights the importance of investigating data variability of a biogenic carbonate paleotemperature proxy at the species level, before applying paleotemperature equations and interpreting the outcome.

## 1. INTRODUCTION: A SINGLE LOCALITY TEST CASE

Since the advent of accurate microdrilling techniques about fifteen years ago, fish otolith stable isotope geochemistry has become a promising new proxy within the field of paleoclimatology. Fish otoliths or 'ear stones' are biogenic accretionary concretions originally composed of aragonite, that precipitate from the endolymph fluid in the inner ear of bony fishes. Development of their use as a paleotemperature proxy gained significantly from work on recent fish taxa. Several empirical paleotemperature equations were established, describing the relationship between the  $\delta^{18}\text{O}$  composition of otoliths and ambient temperature, providing the stable isotope composition of ambient water ( $\delta^{18}\text{O}_{\text{sw}}$ ) is known (e.g. Patterson et al., 1993; Thorrold et al., 1997; Storm-Suke et al., 2007). Therefore, given their often abundant occurrence in Cenozoic shelf sediments worldwide, paleotemperature records potentially covering short- and long-term climatic shifts and cycles in both greenhouse and icehouse settings can be derived from them (Nolf, 1995; Ivany et al., 2000, 2003; De Man et al., 2004). Moreover, their incremental growth ring pattern allows the combined inference of mean annual and intra-annual (seasonal) temperature variations, presenting an obvious advantage over other biogenic carbonates frequently used for stable isotope paleothermometry such as foraminifera and ostracods (Ivany et al., 2000; Vanhove et al., 2011). Previous work dealing with stable O and C geochemistry of fossil otoliths has been limited so far. Secular paleotemperature trends derived

from bulk and seasonal  $\delta^{18}\text{O}$  variability were discussed by Ivany et al. (2003) for the Paleogene U.S. Gulf Coast and by De Man (2004) for the late Paleogene of the southern North Sea Basin. Based on incrementally sampled otoliths, Ivany et al. (2000) suggested cooler winter temperatures as a cause for the large mollusk turnover across the Eocene/Oligocene interval. Attempts were made to infer paleotemperatures as far back in time as the Jurassic, but unfortunately the species used in these studies lack clear affinities with recent species (Patterson, 1999; Price et al. 2009).

Primordial to the interpretation of any paleoenvironmental proxy based on biogenic accretionary carbonates is to have constraints on basic data variability, i.e. intrataxon data spread and any possible intertaxon offsets. Both may obscure the reliability of a given record when not carefully accounted for. Our objective is to assess these potential pitfalls for stable O and C isotope geochemistry on fossil otoliths of early Paleogene marginal marine settings. To achieve this, we carried out a single locality, multi-species test case. Four similar fossiliferous levels were sampled in a sand and silt quarry in Belgium. From each level, the same three otolith species and two bivalve species were sampled to exclude taxonomical bias between different levels. The age of these levels is early late Ypresian (ca. 50.9 Ma) and falls within the early Eocene climatic optimum (EECO) interval (Zachos et al., 2008; Vanhove et al., 2011). The otoliths belonged to demersal, non-migratory

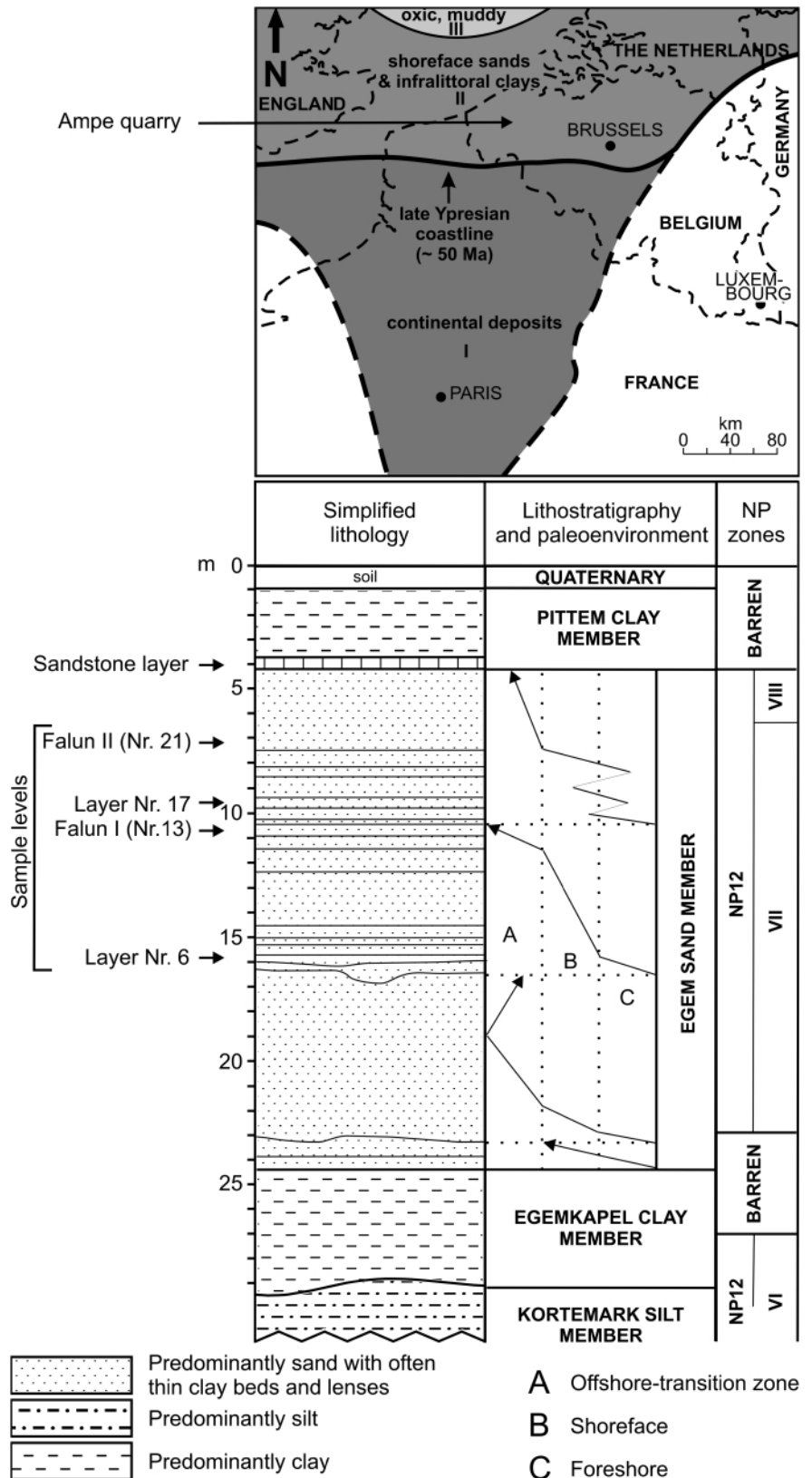
fishes, which are presumably characterized by reduced complexity of their stable isotope signals. Bulk  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were measured and data variability assessed within and between taxa. Results are discussed mainly in terms of  $\delta^{18}\text{O}$  values, as these can be more easily interpreted in terms of paleotemperatures. Although apparent temperature dependent  $\delta^{13}\text{C}$  fractionation was reported in otoliths, the dissolved inorganic carbon (DIC) of a marginal marine environment is even more difficult to constrain than the  $\delta^{18}\text{O}_{\text{sw}}$ , and in addition, substantial metabolic fractionation seems to affect otolith  $\delta^{13}\text{C}$  values (Thorrold et al., 1997; Patterson et al., 1993).

**2. STRATIGRAPHY AND PALEOGEOGRAPHY OF THE AMPE QUARRY**

The Ampe quarry (Fig. 1) is located near the village of Egem, west of the N50 national road between Brugge and Kortrijk, Belgium (51°00'45" N 003°13'56" E). It is the type locality of the Egem Sand Member (Steurbaut and Nolf, 1986). This 20 m thick unit represents the incised valley fill of third order sequence Y-G, and belongs to calcareous nannofossil subzone NP12 (VII), except for its topmost 2 m, which belongs to subzone NP12 (VIII) (Steurbaut, 1998; Vandenberghe et al., 2004). Both subzones are calibrated to magnetochron C23N (Ali et al., 1993). It consists of subhorizontally orientated greenish glauconitic sands often rich in mollusks and nummulitids, with clayey intercalations. The Egem Sand Member was divided by Steurbaut (1998) into 21 layers. Sedimentological features include thin shell lenses and hummocky stratification, indicative of storm-generated sediment reworking and deposition. Several coarsening upward cycles related to small relative sea-level changes, represent deposition in the shoreface to offshore-transition zone (Steurbaut, 2006). Based on a correlation with the Danish Albækhoed section, a total time span of 150 kyr was calculated for the deposition of the member,

with high average sedimentation rates of 41 cm/kyr (Steurbaut, 1998).

Paleogeographically, the Ampe quarry is located within the



**FIGURE 1:** Simplified lithostratigraphic sketch of the Ampe Quarry (after Steurbaut, 2006, Figure 8) and paleogeography of the Ampe Quarry locality (after Vanhove et al., 2011). A,B,C are based on grain size analyses (Steurbaut, 2006).

| Mean $\delta^{13}\text{C}$<br>SD                  | Mean $\delta^{18}\text{O}$<br>SD | Level                   | OID  | $\delta^{13}\text{C}$<br>(‰ VPDB)                          | $\delta^{18}\text{O}$<br>(‰ VPDB)                          |
|---|----------------------------------|-------------------------|--|--|--|
| <b><i>Platycephalus janeti</i></b>                |                                  |                         |  |  |  |
| -5.25<br>0.47                                     | -5.42<br>0.51                    | Falun II                | O15A<br>O15B<br>O15C<br>O15D                       | -5.11<br>-5.90<br>-4.79<br>-5.19                           | -6.01<br>-4.97<br>-5.29<br>-5.97                           |
| -5.51<br>0.21                                     | -4.74<br>0.23                    | Nr. 17                  | O16A<br>O16B<br>O16C<br>O16E<br>O16D               | -5.42<br>-5.21<br>-5.66<br>-5.57<br>-5.72                  | -4.79<br>-5.08<br>-4.67<br>-4.45<br>-4.71                  |
| -5.50<br>0.76                                     | -3.94<br>0.69                    | Falun I                 | O17A<br>O17B<br>O17C<br>O17D<br>O17E               | -6.02<br>-6.03<br>-6.04<br>-4.41<br>-5.00                  | -4.62<br>-4.38<br>-2.86<br>-4.14<br>-3.69                  |
| -5.39<br>0.72                                     | -4.85<br>0.56                    | Nr. 6                   | O18A<br>O18B<br>O18C<br>O18D<br>O18E<br>Mean<br>SD | -6.06<br>-5.71<br>-5.21<br>-4.22<br>-5.73<br>-5.42<br>0.55 | -4.34<br>-4.59<br>-5.68<br>-5.16<br>-4.50<br>-4.73<br>0.75 |
| <b>"genus Neobythitiorum" <i>subregularis</i></b> |                                  |                         |  |  |  |
| -0.93<br>0.52                                     | -2.34<br>0.32                    | Falun II                | O6A*<br>O6B*<br>O6C*<br>O6D*                       | -1.49<br>-0.37<br>-1.25<br>-0.61                           | -2.64<br>-2.44<br>-2.09<br>-2.20                           |
| -1.18<br>0.82                                     | -2.71<br>1.59                    | Nr. 17                  | O31A<br>O31B<br>O31C                               | -1.36<br>-1.89<br>-0.28                                    | -1.80<br>-4.54<br>-1.78                                    |
| -0.56<br>0.91                                     | -2.45<br>1.13                    | Falun I                 | O4A*<br>O4B*<br>O4C*<br>O4D*                       | -1.49<br>-0.41<br>0.64<br>-0.99                            | -1.85<br>-2.26<br>-1.59<br>-4.10                           |
| -0.89<br>0.25                                     | -2.40<br>0.05                    | Nr. 6                   | O33D<br>O33E<br>Mean<br>SD                         | -0.71<br>-1.07<br>-0.87<br>0.67                            | -2.43<br>-2.37<br>-2.47<br>0.88                            |
| <b><i>Paraconger papointi</i></b>                 |                                  |                         |  |  |  |
| -2.55<br>0.70                                     | -3.97<br>0.98                    | Falun II                | O5A*<br>O5B*<br>O5E*<br>O5D*                       | -2.42<br>-3.36<br>-2.73<br>-1.68                           | -5.37<br>-3.90<br>-3.35<br>-3.26                           |
| -3.59<br>0.76                                     | -4.23<br>1.34                    | Nr. 17                  | O30A<br>O30B<br>O30C                               | -4.08<br>-3.97<br>-2.72                                    | -2.89<br>-4.23<br>-5.57                                    |
| -3.14<br>0.51                                     | -4.36<br>0.78                    | Falun I                 | O3A*<br>O3B*<br>O3C*<br>O3D*                       | -3.50<br>-3.70<br>-2.74<br>-2.64                           | -4.11<br>-4.68<br>-5.65<br>-3.02                           |
| -3.67<br>0.46                                     | -3.93<br>0.90                    | Nr. 6<br>Nr. 6<br>Nr. 6 | O32A<br>O32B<br>O32C<br>Mean<br>SD                 | -4.00<br>-3.86<br>-3.14<br>-3.18<br>0.72                   | -3.12<br>-3.78<br>-4.90<br>-4.13<br>0.97                   |
| <b><i>Venericardia sulcata</i></b>                |                                  |                         |  |  |  |
| 1.51<br>0.62                                      | -4.53<br>0.29                    | Falun II                | B3A<br>B3B<br>B3C<br>B3D                           | 0.90<br>1.34<br>2.38<br>1.41                               | -4.74<br>-4.53<br>-4.11<br>-4.73                           |
| 1.79<br>0.26                                      | -4.25<br>0.35                    | Nr. 17                  | B2A<br>B2B<br>B2C<br>B2D<br>B2E                    | 1.87<br>1.57<br>1.63<br>2.22<br>1.67                       | -4.65<br>-3.90<br>-4.10<br>-4.02<br>-4.61                  |
| 1.74<br>0.37                                      | -4.71<br>0.65                    | Falun I                 | B1A<br>B1B<br>B1C<br>B1D<br>B1E                    | 2.05<br>1.53<br>1.96<br>1.97<br>1.18                       | -4.85<br>-3.65<br>-5.11<br>-5.33<br>-4.62                  |
| 0.22  | -4.24                            | Nr. 6                   | B4A<br>Mean<br>SD                                  | 0.22<br>1.63<br>0.55                                       | -4.24<br>-4.46<br>0.53                                     |
| <b><i>Callista laevigata</i></b>                  |                                  |                         |  |  |  |
| 0.62  | -4.73<br>0.85                    | Falun II                | B7A<br>B7C<br>B7D                                  | -0.13<br>0.58<br>1.11                                      | -5.66<br>-3.99<br>-4.54                                    |
| 0.56<br>0.41                                      | -4.72<br>0.80                    | Nr. 17                  | B6A<br>B6B<br>B6C<br>B6D<br>B6E                    | 0.23<br>0.16<br>1.07<br>0.43<br>0.92                       | -4.37<br>-4.49<br>-4.61<br>-6.10<br>-4.06                  |
| 0.96<br>0.33                                      | -4.40<br>0.34                    | Falun I                 | B8A<br>B8B<br>B8C<br>B8D<br>B8E                    | 1.04<br>0.64<br>1.48<br>0.73<br>0.91                       | -4.29<br>-4.96<br>-4.18<br>-4.46<br>-4.09                  |
| 0.42<br>0.28                                      | -3.98<br>0.12                    | Nr. 6                   | B5A<br>B5B<br>B5C<br>B5D<br>B5E<br>Mean<br>SD      | 0.27<br>0.27<br>0.27<br>0.91<br>0.38<br>0.63<br>0.41       | -3.97<br>-4.11<br>-3.88<br>-3.84<br>-4.10<br>-4.43<br>0.60 |

Belgian Basin, i.e. the eastern shallow embayment of the southern North Sea Basin (NSB). At the time of deposition of the Egem Sand Member, this area represented the near-shore rim of the basin (Fig. 1). The presence of nummulitids argues for a connection with southern realms, probably via a south-western connection with the Atlantic Ocean (King, 2006).

### 3. METHODS

About 20 kg of sediment was sampled from each of the four fossiliferous levels along the east side of the Ampe quarry. These correspond from base to top to levels Nr. 6, Falun I (Nr. 13), Nr. 17 and Falun II (Nr. 21) of Steurbaut (2006: Fig. 8). The sediments were wet-sieved at mesh widths 1.000, 0.710 and 0.495 mm. A total of 351 otoliths was picked, 30 of which were used for isotope analysis. Another set of 16 otoliths (indicated by an \* in Table 1) was retrieved from the collections of the Royal Belgian Institute of Natural Sciences, Brussels (RBINS), currently curated by Dirk Nolf. The following three otolith and two bivalve taxa were selected: *Platycephalus janeti* (Priem, 1911) (Platycephalidae, flathead fishes), *Paraconger papointi* (Priem, 1906) (Congridae, conger eels), "genus Neobythitiorum" *subregularis* (Schubert, 1916) (Ophidiidae, cusk-eels), *Venericardia sulcata* (Solander, 1766) and *Callista laevigata* (Lamarck, 1806) (Fig. 2). Quotation marks and the prefix *genus* in otolith taxonomy refer to the affinity of the species with a recent taxon (Nolf, 1985).

In general, otoliths powders were prepared as described in Vanhove et al. (2011). Small otoliths, particularly most *P. janeti* specimens, and bivalves were crushed to fine powders in an agate mortar and homogenized. Stable O and C isotopes were measured at the Stable Isotope Lab of the Free University of Brussels with a ThermoFinnigan Kiel (III) automated carbonate extraction device, coupled to a ThermoFinnigan Delta<sup>plus</sup>XL dual inlet isotope ratio mass spectrometer. For each 4 to 5 samples a NBS-19 standard was measured to calculate analysis precision, which is on average +/- 0.03‰ for  $\delta^{13}\text{C}$  and +/- 0.07‰ for  $\delta^{18}\text{O}$ . Results are reported in  $\delta$ -notation, relative to VPDB. Statistical analyses were performed with Statistica 8.0 (StatSoft). Levene's test was used to test equality of variances between two groups. The unpaired t-test was applied to test whether or not the mean values of the groups differ significantly. In case of unequal variances, the Welch's t-test was used ( $\alpha \leq 0.05$  in all tests).

### 4. RESULTS

The basic, intrataxon variability of the otolith and bivalve data is approximately 2.50 to 3.00‰ for both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ , and is more or less similar for each species and each stratigraphic level (see Table 1 and Fig. 3). Otolith data plot in the lower

**TABLE 1:** Data of otolith and bivalve stable O and C analyses used in this study, with means and standard deviations (SD) calculated per level. Column OID represents identification codes of the samples. Data marked with an \* are sampled from the collections of the RBINS, curated by Dirk Nolf.

left quadrant of the  $\delta^{13}\text{C}$  vs.  $\delta^{18}\text{O}$  cross-plot, while the bivalve data plot in the lower right. For  $\delta^{13}\text{C}$ , all species means differ statistically from each other (t-test  $p=0.000$  in all cases). Hence, the data plot as distinct groups on the cross-plot, with an overall range of  $\sim 8.50\text{‰}$  and an offset of  $4.60\text{‰}$  between the mean values of the three otolith groups. The  $4.50\text{‰}$  overall range in  $\delta^{18}\text{O}$ , with an offset of  $2.20\text{‰}$  between mean otolith values, is smaller than in  $\delta^{13}\text{C}$  values. Offsets in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were observed in each of the four stratigraphic levels sampled (see Table 1). For example, for the otolith specimens from Layer 6, the total offset is  $4.50\text{‰}$  for  $\delta^{13}\text{C}$  and  $2.45\text{‰}$  for  $\delta^{18}\text{O}$ .

The mean  $\delta^{18}\text{O}$  values of *P. janeti* and *P. papointi* otoliths cannot be distinguished statistically (t-test  $p=0.053$ ), and the same holds for the means of the two bivalves *V. sulcata* and *C. laevigata* (t-test  $p=0.789$ ). Moreover, the  $\delta^{18}\text{O}$  data of *P. janeti* and *P. papointi* combined correspond to the bivalve  $\delta^{18}\text{O}$  data (t-test  $p=0.888$ ). "*N.*" *subregularis*  $\delta^{18}\text{O}$  data can be distinguished from every other group (t-test  $p=0.000$  in all cases).

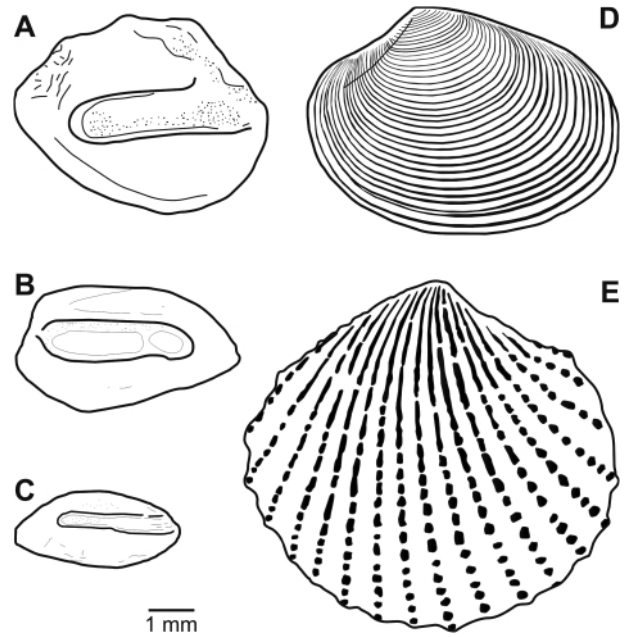
## 5. DISCUSSION

### 5.1 SAMPLE PRESERVATION

During sampling, attention was paid to select only well-preserved specimens. Vanhove et al. (2011) evaluated the preservation of equally well-preserved otoliths as used in this study, including specimens of the Ampe quarry. This work included x-ray diffraction, scanning electron microscopy and cold cathodoluminescence of saggital sections. No compositional or structural differences were detected between species, excluding preferential diagenesis of one or more taxa. Moreover, all samples studied by Vanhove et al. (2011) showed chemical and ultrastructural properties similar to those of the pristine aragonite of recent otoliths. These findings agree with the observation that recrystallization of otolith aragonite into calcite is exceptional (Nolf, 1995), and with earlier reports of well-preserved Oligocene to Pliocene otoliths (Dufour et al., 2000; Woydack and Morales-Nin, 2001). Considering that the aragonite of the otoliths used in this study is indeed pristine, we assume that the original stable isotope composition is preserved (Marshall, 1992).

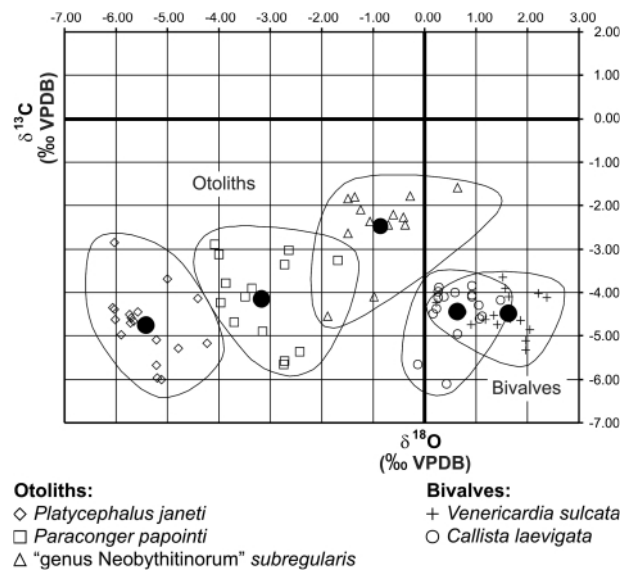
### 5.2 INTRATAXON VARIABILITY

A scatter of  $3.00\text{‰}$  per species for both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  is in general agreement with earlier observations on Jurassic fish otoliths (Patterson, 1999; Price et al., 2009). Our study, focusing on a more recent time interval, has the advantage that the taxa have clear affinities with modern relatives (Nolf, 1985). For example, the genera *Platycephalus* and *Paraconger* are still extant. The three selected taxa belong to non-migratory demersal fishes. Consequently, the data spread is unlikely to result from vertical and horizontal migrations in the water column. According to the taphonomic principle of Nolf (1995), most of the otoliths that arrive in the sedimentary record are derived from the excretion products of marine (migrating) predators. Etching of otoliths is regarded as main indication for



**FIGURE 2:** Otolith (A,B,C, after Steurbaut and Nolf, 1991) and bivalve (D,E) species used in this study. A) *Paraconger papointi*, B) "genus *Neobythitinerum*" *subregularis*, C) *Platycephalus janeti*, D) *Callista laevigata*, E) *Venericardia sulcata*.

this (Nolf, 1985). The assumption that potentially some components of the fauna are allochthonous, probably representing waters with varying salinities due to changes in runoff or evaporation, could partly explain the observed spread. Another likely factor is the fact that each sampled layer represents a death assemblage of otoliths spanning an expanded time interval (several kyr based on sedimentation rates; Steurbaut, 1998). The sedimentological nature of the Egem Sand Mem-



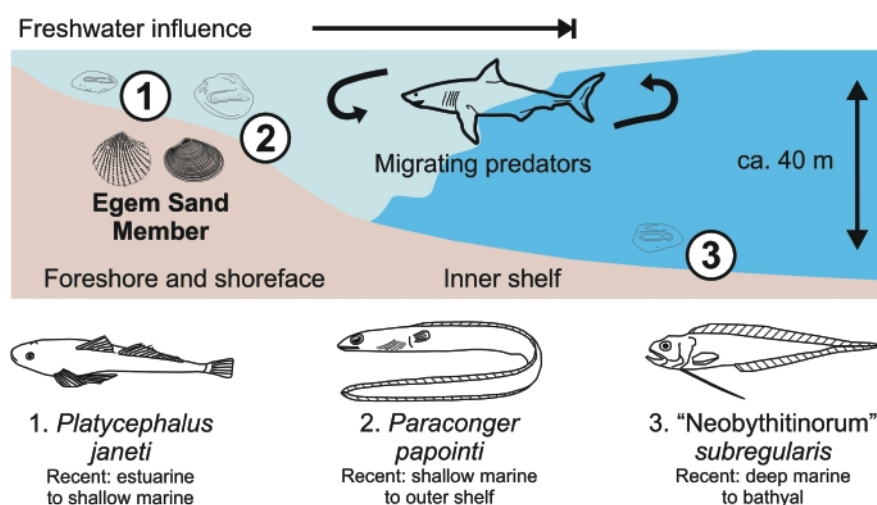
**FIGURE 3:**  $\delta^{13}\text{C}$  vs.  $\delta^{18}\text{O}$  cross-plot of all data used in this study. Arbitrary contours of the five taxa used were drawn to emphasize the extent of data variability. Thick black dots represent the means of individual taxa. Otolith data show three clearly distinguishable groups, due to the large spread of  $7.00\text{‰}$  in  $\delta^{13}\text{C}$ . Considering  $\delta^{18}\text{O}$ , the values of *Platycephalus janeti* and *Paraconger papointi* correspond to the two bivalve data clouds.

ber, indicating local winnowing and reworking due to currents and storm events, supports the latter factor (see section 2; Steurbaut, 2006). Small-scale early post-depositional reworking is also supported by the data spread in both *V. sulcata* and *C. laevigata* bivalves, which approximately equals otolith scatter. At present, it is impossible to discriminate whether temperature, salinity fluctuations or a combination of both, contributed to the scatter in  $\delta^{18}\text{O}$  values. The data spread in other studies using *P. papointi* and "N." *subregularis* otoliths is slightly smaller (e.g. up to 2.50‰ in De Man et al., 2004), suggesting that sample levels in these studies suffered less from taphonomic processes. In conclusion, according to our data, the combination of temporal environmental variability, post-mortem transport and local reworking, poses a complicating factor to otolith stable isotope paleothermometry in marginal marine basins, because a scatter of 3.00‰ would mean a  $\sim 12$  °C temperature uncertainty using any available paleotemperature equation established on otoliths (e.g. Thorrold et al., 1997).

### 5.3 INTERTAXON VARIABILITY

As measured  $\delta^{18}\text{O}$  values are mainly a function of ambient temperature and the isotopic composition of the surrounding sea water, temperature gradients and salinity fluctuations can be regarded as primary causes of the observed intertaxon offsets. The spread in mean oxygen isotope values of the three otolith groups would translate in a gradient of ca. 10–12 °C: such a temperature gradient in the basin is unrealistic, because we used otoliths of fishes with a benthic mode of life. Furthermore, the sedimentology of upper Ypresian deposits of the proximal Belgian Basin indicates well-oxygenated conditions and strong currents, with depths not exceeding 10–20 m, arguing against stratification of the water column (see section 2; Steurbaut, 2006). Deviation from average oceanic  $\delta^{18}\text{O}_{\text{sw}}$  values in marginal basin waters on the other hand, presents a

common problem to the interpretation of  $\delta^{18}\text{O}$  isotope signals measured on biogenic carbonates in these areas (e.g. Andreasson and Schmitz, 1996; Ivany et al., 2004; Tindall et al., 2010). Few studies addressed the possibility of freshwater mixing with marine North Sea Basin waters during the Ypresian (Schmitz et al., 1996; Zacke et al., 2009). We believe that freshwater influence is indeed the most likely explanation for the observed intertaxon offset in  $\delta^{18}\text{O}$  values of otoliths. Our hypothesis is based on three arguments, and comparison with bivalve data (Fig. 4). Firstly, deposition of the Egem Sand Member at the Ampe quarry location occurred close to the coast. It is very likely that this site was seasonally or continuously influenced by freshwater mixing, as is the case with modern shallow margins of the North Sea Basin (Harwood et al., 2008). Secondly, modern nearest relatives of "N." *subregularis* thrive in outer neritic to bathyal depths, while for *P. janeti* and *P. papointi* this is inner neritic (Böhlke et al., 1989; Nolf, 1995; Nielsen et al., 1999). Notwithstanding a potential shift of "N." *subregularis* in habitat preference through time towards deeper realms, this suggests that during the Ypresian "N." *subregularis* may have preferred more open marine conditions compared to the other two species. Thirdly, the taphonomic principle of Nolf (1995) indicates that most of the otoliths in an assemblage underwent post-mortem transport before deposition. In summary, according to our model, the sampled layers in the Egem Sand Member each represent a thanatocoenosis consisting of locally deposited otoliths (e.g. *P. janeti* and *P. papointi*) and otoliths transported coastwards by migrating predators originating from more distal realms (e.g. "N." *subregularis*). This explains the very negative  $\delta^{18}\text{O}$  values for *P. janeti* and *P. papointi*, and the more positive values for "N." *subregularis*. The  $\delta^{18}\text{O}$  data of the coastal bivalve species *V. sulcata* and *C. laevigata*, confirm this hypothesis. These were deposited relatively in situ, and hence should have incorporated local  $\delta^{18}\text{O}_{\text{sw}}$  signals. For *Venericardia*, supposed equilibrium deposition was demonstrated before (see discussion in Ivany et al., 2004). The mean of all bivalve  $\delta^{18}\text{O}$  data in our study statistically corresponds to the mean of the combined group *P. janeti* - *P. papointi*, suggesting that the latter taxa indeed bear coastal stable isotope signals, while "N." *subregularis* does not. Accordingly, because "N." *subregularis* likely thrived in waters with  $\delta^{18}\text{O}_{\text{sw}}$  values closely related to open oceanic values,  $\delta^{18}\text{O}$  measurements on otoliths of this species seem well-suited for paleotemperature derivations. Our hypothesis is also compatible with the observed pattern in otolith  $\delta^{13}\text{C}$  values. As the  $\delta^{13}\text{C}$  of otoliths partly represents environmental DIC, the range in mean



**FIGURE 4:** Graphic representation of the freshwater influx hypothesis (see text for explanation). Numbers in circles represent the paleohabitats of the three fish species of which modern closest representatives are pictured in the lower half of the graph. Light grey: freshwater influence zone; dark grey: water of normal salinity; middle grey: sediments. Horizontal range of the field of view is ca. 30 km.

$\delta^{13}\text{C}$  values from  $-5.50\text{‰}$  in *P. janeti* towards  $-1.00\text{‰}$  in “N.” *subregularis*, probably reflects the degree of influence of  $\delta^{13}\text{C}$  depleted (riverine) water on the three species, from a relatively large to smaller influence, respectively.

Physiological effects and variability in biogenic carbonate production are two other factors that may further explain observed intertaxon discrepancies. The first is known to cause non-equilibrium incorporation of elements and isotopes in biogenic minerals with respect to ambient water signatures (Weiner and Dove, 2003). This likely presents an additional explanation for the large range in  $\delta^{13}\text{C}$  values of the otolith data, as in otoliths metabolic overprinting of environmental  $\delta^{13}\text{C}$  DIC values, probably related to somatic growth and precipitation rate, causes substantial departures from equilibrium (Kalish, 1991; Thorrold et al., 1997). In addition, incorporation of metabolic carbon may also explain the heavier  $\delta^{13}\text{C}$  values of the bivalve species compared to those of all three otoliths species. Bony fish and bivalve mollusks are taxonomically very distant to each other, and both have their own intricacies with respect to the incorporation of  $\delta^{13}\text{C}$  signals into their carbonate precipitate (e.g. for bivalves: Gillikin et al., 2007; McConnaughey and Gillikin, 2008). Positive  $\delta^{13}\text{C}$  values of  $\sim 1\text{--}2\text{‰}$  for *Venericardia sulcata* bivalves are in line with *Venericardia imbricata* values of the middle Eocene the Paris Basin (Andreasson and Schmitz, 1996). True equilibrium precipitation of  $\delta^{18}\text{O}$  in otoliths is disproven by the establishment of several paleotemperature equations, based on different taxa (Kalish, 1991; Patterson et al., 1993; Thorrold et al., 1997; Høie et al., 2004; Storm-Suke et al., 2007; Dorval et al., 2011). Despite errors that may result from different methods or experimental setups, and keeping in mind different calibration ranges, the equations show considerable variation of their intercepts. Except for the equations of Kalish (1991) and Dorval et al. (2011), slopes are similar, suggesting that fish precipitate  $\delta^{18}\text{O}$  with offsets that are taxon-specific but which remain constant with changing temperature. All relationships reflect precipitation “close to equilibrium”, and  $\delta^{18}\text{O}$  may even be precipitated in equilibrium with respect the endolymph fluid (see introduction). However, in the field of quantitative paleothermometry the “small” departures from equilibrium can be regarded as disequilibrium precipitation, particularly because the equations published up to now cover a  $\sim 9\text{ °C}$  temperature range for a given  $\delta^{18}\text{O}$  value. The reasons for this are still unclear, but subtle metabolic differences between species seems the most likely explanation (Kalish, 1991; Storm-Suke et al., 2007). Measurements on *P. papointi* and “N.” *subregularis* otoliths of early Lutetian age in the Belgian Basin and of Ypresian age in the U.S. Gulf Coastal Plain, do not indicate interspecific discrepancies in  $\delta^{18}\text{O}$ , implying that the offsets observed in our study are caused by a temporal phenomenon (De Man et al., 2004; Ivany et al., 2003). This, however, is not in agreement with a different departure from equilibrium precipitation between these two species, since one would expect such offsets to be constant through time.

The second factor, variability in carbonate production, may

lead to irregularities of the incremental pattern of otoliths (e.g. Pannella, 1980). For example, because of varying metabolic rates, during one season more carbonate may be produced, resulting in thicker growth rings relative to another, even in tropical fishes (Henderson, 2006). To assess whether such effect could have caused intertaxon offsets in  $\delta^{18}\text{O}$  values, patterns arising from a set of incremental stable O and C isotope data of *P. papointi* and “N.” *subregularis* otoliths were evaluated (Vanhove et al., 2011). These patterns represent a clear seasonal signal, corresponding to visual growth bands. Wavelengths of both seasons are approximately equal within a species. There is also no evidence of a distinct effect related to the larval, planktonic stage of the fishes. Based on these data, there are no indications of substantial intertaxon differences in the way otoliths precipitate their carbonate, influencing our data.

The similarity in seasonal amplitudes between *P. papointi* and “N.” *subregularis* in Vanhove et al. (2011), both on average  $\pm 1\text{‰}$ , may seem contradictory to our freshwater influence hypothesis, since one could expect larger amplitudes in otolith  $\delta^{18}\text{O}$  values of specimens with a more proximal habitat. However, this depends on the nature of the presumed  $\delta^{18}\text{O}_{\text{sw}}$  depleted water masses, which could have been either seasonal or continuous. For example, continuous influence would imply that pronounced seasonal temperature contrasts of  $\sim 9.5\text{ °C}$  prevailed in the region, and that this was recorded by both *P. papointi* and “N.” *subregularis* (Vanhove et al., 2011). Ivany et al. (2003) reported smaller seasonal ranges of temperature variation in middle Eocene “Lepophidiinarum” (Ophidiid; same family as “N.” *subregularis*) otoliths the U.S. Gulf Coast, compared to *Paraconger* otoliths. This could be indicative of a more distal habitat preference of Ophidiids relative to Congrids, but such a small ranges were not observed by Vanhove et al. (2011). Unfortunately, both the observations of Ivany et al. (2003) and Vanhove et al. (2011) are based on a very limited number of incremental patterns. Further discussion on this, involving a profound evaluation of inferences from other proxies and paleotemperature interpretations, is beyond the scope of this paper and an objective for additional investigation.

## 6. CONCLUSIONS AND IMPLICATIONS

A total offset of  $4.60\text{‰}$  in  $\delta^{13}\text{C}$  and  $2.20\text{‰}$  in  $\delta^{18}\text{O}$  between the mean values of the three otolith species used is observed. This discrepancy is characteristic of each of the four levels sampled in the Ampe quarry, presenting a strong case for intertaxon variability at the species level within otolith stable O and C isotope data. Freshwater influence on coastal waters seems the most plausible explanation, and is supported by analyses on coastal bivalve species and information on recent relatives of the fish taxa used. The observed  $2.50\text{--}3.00\text{‰}$  intra-taxon variability is in agreement with earlier otolith data, and a combination of temporal and taphonomic effects is interpreted here as a likely cause. The results stress the importance of assessing data variability at the species level, preferably by means of a single-locality, multilevel test case. They imply that within a single taphonomic setting of a marginal marine ba-

sin, some otolith taxa, in our case "N." *subregularis*, seem well-suited for  $\delta^{18}\text{O}$  stable isotope paleothermometry, while others may be strongly biased by continental run-off. Some of the few earlier studies on otolith paleothermometry briefly mentioned the potential pitfalls of taphonomy in this type of research, nevertheless interpretation of the secular data series in these studies would benefit from a more robust approach towards intra- and interspecific data variability (e.g. Ivany et al., 2003; De Man et al., 2004). In order to increase the resolution and precision of paleotemperature calculations from otolith stable O and C isotope data, based on our data such an approach is a necessary step to take before paleotemperature equations are applied. Future research directions include similar analyses on other otolith taxa, the incorporation of data from other localities and time frames, and alternative testing of the freshwater hypothesis by means of clumped isotope geochemistry.

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