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Interpretation of benthic foraminiferal stable isotopes in subtidal estuarine environments

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Abstract

Here we present a novel approach for the interpretation of stable isotope signatures recorded in benthic foraminifera from subtidal estuarine environments. The stable isotopic composition (δ^{18}O and δ^{13}C) of living *Ammonia tepida* and *Haynesina germanica* is examined in four stations in the Auray River estuary (Gulf of Morbihan, France) sampled in two contrasting seasons, spring 2006 and winter 2007. Comparing benthic foraminiferal δ^{18}O measurements with theoretical oxygen isotopic equilibrium values, calculated on the basis of water temperature and salinity measurements in the upper and lower estuary, i.e., T-S-$\delta^{18}O_{eq.}$ diagrams, strongly suggests that foraminiferal faunas sampled at the four stations calcified during different periods of the year. This interpretation can be refined by using the benthic foraminiferal δ^{13}C which is mainly determined by the mixing of sea and river water. In the upper estuary foraminifera mainly calcified in early spring and winter, whereas in the lower estuary calcification took mainly place in spring, summer and autumn. This new method provides insight into the complexity of estuarine benthic foraminiferal stable isotope records. In addition, it can also be used to obtain new information on preferred calcification periods of benthic foraminiferal taxa in different parts of the estuary.

1 Introduction

Benthic foraminifera from shallow water settings can be successfully used as proxies of environmental variables (e.g., Redois and Debenay, 1996; Debenay et al., 2000; Duchemin et al., 2005; Murray, 2006) or as a bio-indicators of the ecological status of the environment (e.g., Alve, 1995; Debenay et al., 2001; Morvan et al., 2004; Armynot du Châtelet et al., 2004; Platon et al., 2005; Le Cadre and Debenay, 2006; Bouchet et al., 2007). However, the use of foraminifera in such settings is often hampered by extreme spatial and temporal variability of the foraminiferal faunas. Detailed studies of benthic foraminiferal faunas from intertidal and subtidal environments show a high

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degree of patchiness (Hohenegger et al., 1993; Murray and Alve, 2000; Swallow, 2000; Hippensteel et al., 2000; Buzas et al., 2002; Morvan et al., 2006). High-frequency studies (over extended periods, i.e., more than a year) indicate that the temporal variability of the faunas may be important (Boltovskoy and Lena, 1969; Cearreta, 1988; Swallow, 2000; Murray, 2000; Alve and Murray, 2001; Buzas et al., 2002; Morvan et al., 2006). This spatial and temporal variability concerns the overall faunal density as well as the density of individual taxa, both varying in an unpredictable way.

Subtidal estuarine environments are affected by strong temperature and salinity variations throughout the year. The impact of these parameters may vary between different parts of the estuary with salinity changes being more important in the upper parts than in the lower parts. Because both temperature and $\delta^{18}O_w$ (mainly related to salinity) have a major impact on $\delta^{18}O$ of carbonate shells, large temporal variations in these parameters make it difficult to interpret benthic foraminiferal $\delta^{18}O$ in subtidal estuarine environments. Salinity also influences the carbon isotopic composition of foraminiferal shells because dissolved inorganic carbon in river water has generally lower $\delta^{13}C_{DIC}$ values compared to open ocean waters (e.g., Bauch et al., 2004). In addition, the degradation of isotopically light organic carbon may locally deplete the $\delta^{13}C$ signature recorded in the carbonate of the benthic foraminiferal shells in relation to bottom water $\delta^{13}C_{DIC}$ (e.g., Chandler et al., 1996). The specific features of these environments, together with the complexity of ecological factors outlined above (i.e., patchiness, strong variability in reproductive periods) complicate the applicability of oxygen foraminiferal stable isotopes in paleoenvironmental reconstructions.

In the present study we aim at better understanding the parameters influencing oxygen and carbon isotopic composition of subtidal estuarine benthic foraminifera from the Auray River estuary (Gulf of Morbihan, France). The $\delta^{18}O$ and $\delta^{13}C$ isotopic composition of living specimens of *Ammonia tepida* (Cushman) and *Haynesina germanica* (Ehrenberg) is documented at four stations sampled during spring 2006 and winter 2007. Foraminiferal stable isotope signatures are difficult to interpret because of the substantial variations in bottom water temperature and salinity throughout the year.

Here, we propose a novel approach to interpret stable isotopes in such environments based on the comparison of foraminiferal isotopic measurements with the annual temperature and salinity cycle in different parts of the estuary. This approach provides valuable information about the timing of foraminiferal calcification and will improve our understanding of stable isotope records based on fossil estuarine assemblages.

2 Study area

The Auray River estuary is located north of the Gulf of Morbihan (Southern Brittany, France, Fig. 1), a semi-enclosed embayment. Fresh water input to the estuary mainly comes from two rivers: Loc'h and Bono (Fig. 1). These waters mix with marine waters of the Gulf of Morbihan originating from the Bay of Quiberon and entering through the Straits of Port Navalo (Fig. 1). The mean tidal range varies between 2.90 m and 4.10 m. The sediments in the outer areas of the Auray river estuary (e.g., Locmariaquer) are composed of sand with low organic carbon content, whereas within the Auray estuary, sediments of areas influenced by tidal currents are generally enriched in the fine fraction and organic matter (Redois and Debenay, 1996). Phytoplankton production is higher in the lower estuary than in the upper estuary and is mainly composed of diatoms (benthic, fresh water or pelagic/oceanic, depending on the season, Paulmier, 1972). In the lower estuary two annual primary production maxima are observed in May and September, whereas in the upper estuary maximum production is restricted to May and June.

3 Material and methods

3.1 Bottom water samples

Bottom water temperature and salinity were monitored every 10 min between March 2006 and March 2008 with a 600 OMS YSI Sensor located 10 cm above the bottom at

two subtidal stations in the Auray River estuary (Fig. 1), (except for sensor sporadic failure). Daily average values were calculated to characterise the annual cycle of bottom water temperature and salinity in the upper (Bono station) and lower (Locmariaquer station) estuary (Fig. 1).

Stable isotopes of bottom waters ($\delta^{13}C_{DIC}$ and $\delta^{18}O_w$) were measured on eighteen samples collected at the Bono site (Fig. 1) every 2 h between the 1st and 3rd of March 2007. Water samples from the sediment-water interface were stored in 12 mL Exetainer vials, poisoned with 20 µL of saturated mercuric chloride ($HgCl_2$) solution, capped tightly and stored in a cool room until analysis. The $\delta^{18}O$ of water and $\delta^{13}C$ of dissolved inorganic carbon (DIC) was measured at the Earth System Science laboratory of Vrije Universiteit Brussel (Belgium) using the protocol described by Gillikin and Bouillon (2007). For $\delta^{18}O_{water}$ measurements, 500 µL of a water sample and 1000 µL pure CO_2 from a tank were injected in a 12 mL helium-flushed headspace vial. After about 48 h equilibration in a shaker at ambient laboratory temperature (23°C), 500 µL of CO_2 from the headspace was injected into the carrier gas stream of a ThermoFinningan Delta XL continuous flow IRMS. Two in-house standards well calibrated against VSMOW were processed with the same protocol. Data are expressed in ‰ relative to VSMOW and precision was better than 0.12‰ (1σ) determined by repeated analysis of the standard. For the $\delta^{13}C_{DIC}$ measurements, 9.5 mL of water sample was acidified with 200 µL of pure orthoposphoric acid in a 12 mL helium-flushed headspace vial. After overnight equilibrium, 500 µL of the headspace was injected into the carrier gas stream of the IRMS (like in $\delta^{18}O_{water}$ analysis). The Miyajima et al. (1995) formula was used to correct for the partitioning of CO_2 between headspace and water phase and to calculate $\delta^{13}C_{DIC}$. Data are expressed in ‰ relative to VPDB and precision was better than 0.15‰ (1σ) based on 14 analysis CO_2 reference gas.

3.2 Foraminiferal samples

3.2.1 Sampling sites

Four subtidal stations (Kerdaniel, Kerdréan, Moustoir and Locmariaquer) on a transect along the Auray River estuary were selected for this study (Fig. 1). Kerdaniel (47°38′51.92″ N, 2°58′18.127″ W) is located in the upper estuary slightly upstream of the Bono River outlet. Kerdréan (47°37′392″ N, 2°57′187″ W) and Moustoir (47°36′08.366″ N, 2°57′27.080″ W) characterize the middle/upper and middle/lower part of the estuary, respectively, whereas Locmariaquer (47°34′12.737″ N, 2°56′35.190″ W) is located at the mouth of the estuary and represents the most open marine conditions. Each station was sampled in spring 2006 (20–21 April or 9 May) and winter 2007 (2 February). During sampling, two sediment cores (replicate samples 01 and 02, a few meters apart) were collected at each station. All samples recovered in February 2007 as well as the samples taken at Kerdaniel and Moustoir in spring 2006 were recovered with a squared gravity corer (15 cm × 15 cm). Samples taken in spring 2006 at Kerdréan and Locmariaquer were collected by scuba divers that pushed a pvc core (9 cm diameter) into the sediment. In winter 2007, we obtained only a sample of the uppermost centimetre at Locmariaquer. The sediment of Kerdaniel and Moustoir stations is mainly composed of mud with high amounts of plant remains. In Kerdréan, the sediment contains a significant proportion of bioclastic fragments, whereas siliciclastic sands dominate at Locmariaquer. All stations contain variable proportions of meiofauna (mainly polychaeta).

3.2.2 Sample processing

Immediately upon collection, cores were subsampled into slices of 1 cm down to 10 cm depth and subsamples were put into bottles containing ethanol and Rose Bengal (1 g/l). In the laboratory the samples were wet sieved into 63–150 µm and >150 µm size classes, and then stored in 95% ethanol. Living (Rose Bengal stained) foraminifera

from both size fractions were picked from wet samples and stored in micropaleontological slides. We only considered foraminifera alive, when all chambers except the last zero to three were stained bright red/pink. Except for some intervals where the foraminiferal fauna was particularly rich, samples were not split and all living benthic foraminifera were picked and counted. Faunal densities for each core (0–10 cm) are expressed as the total number of foraminifera standardised to a 50 cm^2 surface area.

3.2.3 Isotopic measurements

Well preserved individuals of living benthic foraminifera *Ammonia tepida* and *Haynesina germanica* were picked from the >150 µm fraction of the uppermost centimetre of sediment at the four studied stations, from each replicate core, and for both sampling periods. Stable carbon and oxygen isotope measurements were performed on 6–10 specimens and measurements were carried out at the stable isotope facilities of Utrecht University using an isotope ratio mass spectrometer (Thermo Finnigan MAT-253) coupled online to an automated carbonate sample preparation device (Kiel III). Results are calibrated against international standard NBS-19 and reported on the Vienna PeeDee Belemnite (VPDB) scale. External reproducibility (1σ) was better than ±0.08‰ and ±0.04‰ for $\delta^{18}O$ and $\delta^{13}C$, respectively.

4 Results

4.1 Temperature and salinity

Temperature values in the Auray river waters are remarkably similar between the upper and lower estuary (Fig. 2a). Lowest water temperatures occur during winter (December–March), and range from 3.5°C to 12°C. Warmer temperatures during summer (middle of June to end of August) range from 18 to 25°C. By contrast, the annual salinity cycle in the upper estuary differs substantially from that observed in the lower

parts of the estuary. In the upper estuary salinity varies between 18 and 34 whereas in the lower estuary it only varies between 27 and 36 (Fig. 2b). In the upper parts, high salinities coincide with dry months (late spring–summer), but can extend well into the beginning of autumn (October–November, Fig. 2b). During these periods the salinity gradient between upper and lower estuary is relatively subtle with salinities ranging only from 30 to 36 (Fig. 2b). Lower salinities are recorded in the upper parts of the estuary during periods of increased precipitation and river discharge in winter, early spring, or during exceptionally rainy periods in summer (e.g., June–July 07, Fig. 2b).

4.2 Mixing of river and sea water in the Auray river estuary and impact on stable carbon and oxygen isotopic ratios

Paired measurements of bottom water $\delta^{13}C_{DIC}$, $\delta^{18}O_w$ and salinity at Bono are used here to create a mixing model of sea and river water for the Auray estuary (e.g., Thomas et al., 2000; Polyak et al., 2003; Reinhart et al., 2003; Bauch et al., 2004; Cronin et al., 2005; McGann, 2008). Over the measured salinity range (20–32), both $\delta^{13}C_{DIC}$ and $\delta^{18}O_w$ show a strong linear relation with salinity (Fig. 3). Regression lines for $\delta^{13}C_{DIC}$ and $\delta^{18}O_w$ to salinity are $\delta^{13}C_{DIC}$ (‰ VPDB)=−8.0+0.25×Salinity (n=18, R^2=0.978, p=0.000) and $\delta^{18}O_w$ (‰ VSMOW)=−4.56+0.14×Salinity (n=18, R^2=0.955, p=0.000), respectively. The stable oxygen isotopic composition of the end member (fresh water) is comparable to the oxygen isotope ratios of precipitation in the Loire-Bretagne catchment area (−4.8‰±0.5, GNIP data for Brest Plouzane, yearly average 1996–2002, International Atomic Energy Agency). The intercept of the $\delta^{13}C_{DIC}$: salinity relationship (−8.0‰) is comparable to the average of $\delta^{13}C_{DIC}$ values in the Rhône-Saône Rivers (−11.0‰, Aucour et al., 2003). Depleted river $\delta^{13}C_{DIC}$ values are explained by the reaction of carbon isotopically depleted soil biogenic CO_2 and minerals of the basement rocks. Mixing of sea and river water (i.e., salinity) has a much greater impact on $\delta^{13}C_{DIC}$ than on $\delta^{18}O_w$. According to the mixing model for the Auray estuary, a change of 15 salinity units leads to a 3.7‰ shift in $\delta^{13}C_{DIC}$ and a 2.1‰ shift

in $\delta^{18}O_w$.

4.3 Living benthic foraminiferal faunas

The abundance of live benthic foraminifera (total number of benthic foraminifera >63 µm per 50 cm^2) shows large differences between stations, replicate cores and sampling periods (Fig. 4a). In spring 2006 (April–May), the abundance of live foraminifera is substantially higher at Kerdréan (~400 individuals per 50 cm^2) and at Locmariaquer (~200 individuals per 50 cm^2) than at the other two stations (50 individuals per 50 cm^2). In February 2007 the abundance is relatively uniform along the Auray estuary (~300–500 individuals per 50 cm^2), although values are still maximal at the Kerdréan site (Fig. 4a).

The spatial distribution of the two species used in this study is typical of East Atlantic temperate estuaries of the French and Spanish coast (e.g., Redois and Debenay, 1996; Debenay et al., 2000, 2006; Duchemin et al., 2005; Leorri et al., 2008). *Ammonia tepida* characterizes the upper and middle/upper parts of the estuary (Fig. 4b) whereas *Haynesina germanica* is represented all along the estuary, although it is more abundant in the upper parts (Fig. 4c). There are some differences in the composition of benthic foraminiferal assemblages between spring 2006 and winter 2007. The abundance of *A. tepida* and *H. germanica* is substantially higher in winter 2007 in the upper and middle/upper estuary.

4.4 Stable carbon and oxygen isotopes of *A. tepida* and *H. germanica*

The $\delta^{13}C$ of *A. tepida* and *H. germanica* increases from the upper to the lower part of the estuary both during winter and spring (Fig. 5, Table 1). The $\delta^{13}C$ of *A. tepida* increases on average by 2.4‰ in spring 2006 and by 1.9‰ in winter 2007. Average differences in $\delta^{13}C$ of *H. germanica* between upper and lower estuary are 2.9‰ in spring 2006 and 2.5‰ in winter 2007. The observed spatial range in benthic foraminiferal $\delta^{13}C$ (dashed lines in Fig. 3) agrees well with the calculated $\delta^{13}C_{DIC}$ gradient caused

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by mixing of sea and river water (Fig. 3). Unlike carbon isotopes, oxygen isotope ratios of *A. tepida* and *H. germanica* tests do not show a clear spatial trend. In the upper estuary, the oxygen isotope values of these species show large differences between spring 2006 and winter 2007. Both species show heavier $\delta^{18}O$ values by about 0.8‰ in spring 2006 compared to winter 2007. Surprisingly, in the lower estuary, the $\delta^{18}O$ of *A. tepida* shows rather low values in spring 2006. At Locmariaquer $\delta^{18}O$ is about 1‰ lower than at the other three stations. At the same station, spring 2006 values are about 0.5‰ depleted in comparison to winter 2007 values (Fig. 5). *H. germanica* does not show such a clear $\delta^{18}O$ minimum at Locmariaquer in spring 2006. In the middle estuary oxygen isotope composition of *A. tepida* and *H. germanica* remain fairly similar and do not show substantial changes between sampling periods.

The temperature gradient between upper and lower estuary is negligible at both sampling times (Fig. 2a). Consequently, we expected to find an increasing trend in the $\delta^{18}O$ of benthic foraminifera from upper to lower parts of the estuary in response to decreasing fresh water influence (Fig. 3). This gradient should have been steeper in winter, when the salinity gradient between upper and the lower estuary is at a maximum, and reduced in spring when the salinity gradient is weaker (Fig. 2b). However, this is not observed, which suggests that the oxygen isotopic composition of benthic foraminifera in the Auray estuary does not represent the temperature and $\delta^{18}O_w$ signatures at the time of sampling.

5 Discussion

5.1 Benthic foraminiferal stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) in subtidal environments

High spatial and temporal variability of the physical parameters (temperature and salinity) complicate the interpretation of the isotopic composition of benthic foraminiferal shells in subtidal environments. Moreover, the highly complex ecology of the

foraminiferal faunas excludes a straightforward application of stable isotopes to estimate temperature or salinity variations in subtidal estuaries. However, by combining foraminiferal stable isotopes and the annual Temperature (T) and Salinity (S) cycle, presented together in T-S-$\delta^{18}O_{equilibrium}$ diagrams, it is possible to constrain foraminiferal calcification seasons. Previously, similar diagrams have been used in paleoceanographic studies in open ocean settings to separate water masses (e.g., Zahn and Mix, 1991; Labeyrie et al., 1992; Zahn et al., 1997). Our approach is novel in that we combine the water mass mixing (i.e., salinity) with the annual temperature cycle. This approach is particularly well suited for the interpretation of stable isotopes in shallow water environments.

Theoretical $\delta^{18}O_{eq}$ fractionation lines are calculated from water $\delta^{18}O$ ($\delta^{18}O_w$) and temperature (T) according to the paleotemperature equation of Shackleton (1974): $T=16.9-4.38\ (\delta^{18}O_{eq}-\delta^{18}O_w)+0.1(\delta^{18}O_{eq}-\delta^{18}O_w)^2$. The $\delta^{18}O_w$ values are based on the $\delta^{18}O_w$: salinity relationship described in the results section ($\delta^{18}O_w$ (‰ VSMOW)$=-4.565+0.1416\times$Salinity) for the Auray River estuary. Standard mean ocean water $\delta^{18}O_w$ (VSMOW) was converted to PDB units $\delta^{18}O_w$ (VPDB) using the factor conversion of Hut (1987; −0.27). Subsequently water temperature and salinity measurements (Fig. 2a and b) from Bono and Locmariaquer hydrographic stations (Fig. 1) have been averaged for 15-day intervals, from March 2006 to March 2008. The calculated values have been plotted in the T-S-$\delta^{18}O_{eq}$ diagram. The diagram clearly shows the annual cycle of theoretical $\delta^{18}O_{calcite}$ in the upper (e.g., Fig. 6a) and lower (e.g., Fig. 6e) estuary. The seasonal succession of calculated $\delta^{18}O_{eq.}$ is more differentiated in the upper part of the estuary where large seasonal changes in salinity result in a clear separation between cooler and low salinity winter waters and warmer and higher salinity late spring to autumn waters (e.g., Fig. 6a). Conversely, the seasonal cycle in the lower parts of the estuary is less differentiated and mainly determined by temperature changes (e.g., Fig. 6e).

The $\delta^{18}O$ values of *A. tepida* and *H. germanica* (Table 1) are plotted in the T-S-$\delta^{18}O_{eq}$ diagrams as lines based on the $\delta^{18}O_{eq}$ fractionation which represents unique

combinations of temperature and salinity values. In case several isotopic measurements have been performed for the same sample a "band" representing the range of measured values is shown. The possible calcification periods are indicated by the overlap between these lines/bands and the areas representing the seasonal $\delta^{18}O_{eq}$ signature. Isotopic data from the upper (Kerdaniel) and middle/upper (Kerdréan) estuary are compared with the *T* and *S* data measured at Bono (Fig. 6a–d), whereas isotopic data from the lower estuary (Locmariaquer) are compared to the *T* and *S* values of Locmariaquer (Fig. 6e, f). No comparison is presented for the middle/lower estuary (Moustoir) since *T* – *S* records are not available for this part of the estuary.

The *T*-*S*-$\delta^{18}O_{eq}$ diagrams (Fig. 6) indicate that in many cases there is no unique solution but several calcification periods are feasible. In the upper estuary (Kerdaniel and Kerdreán) *Ammonia tepida* sampled in Spring 2006 and Winter 2007 may have calcified in spring, winter or autumn (Fig. 6a-d). The same applies to *H. germanica* except for the specimens collected in Kerdaniel in winter 2007 which can only have calcified in spring or early summer. In the lower estuary (Locmariaquer) *H. germanica* and *A. tepida* may have calcified in autumn, summer or spring (Fig. 6e, f) with the exception of the specimens collected in spring 2006 which have an isotopic signature typical of summer conditions (Fig. 6e).

The $\delta^{13}C_{DIC}$ values of Auray River water show a positive linear relation to salinity (Fig. 3). The observed spatial trend in benthic foraminiferal $\delta^{13}C$ agrees well with the expected $\delta^{13}C_{DIC}$ gradient caused by mixing of sea and river waters (Fig. 3). Therefore, by combining *T*-*S*-$\delta^{18}O_{eq}$ diagrams with additional salinity constraints provided by carbon isotopes (last column in Table 2) we can reduce the number of possible calcification periods.

Subtidal areas of the Auray River estuary are characterized by organic rich sediments. It can therefore not be excluded that oxidation of isotopically light organic matter contributes to the carbon isotopic composition of *A. tepida* and *H. germanica* shells. However, the average spatial gradient of foraminiferal $\delta^{13}C$ (around 2.9‰, Table 1) between the upper and lower estuary corresponds to the expected $\delta^{13}C_{DIC}$ gradient in

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function of a 10 salinity change (Fig. 3). Therefore, the contribution of ^{13}C depleted DIC by organic matter degradation is probably small in our case. Consequently, although it is not possible to directly translate $\delta^{13}C$ into a salinity value, we can use the carbon isotopic signature to rule out some of the theoretically possible calcification periods in the T-S-$\delta^{18}O_{eq}$ diagram.

In the upper estuary (Kerdaniel and Kerdreán) calcification during autumn can be excluded for both sampling periods and both species because their $\delta^{13}C$ indicates low salinity during calcification. Consequently, both species can only have calcified in winter or early spring. The restricted salinity range in the lower parts of the estuary makes the distinction between spring and autumn calcification more difficult (Fig. 6e, f). Although the carbon isotopic signatures are in line with the observations in the T-S-$\delta^{18}O_{eq}$ diagram we can not use them to further constrain calcification periods.

A summary of the most likely timing of foraminiferal calcification in the Auray estuary is presented in Table 2. In the upper estuary both species calcified in winter or early spring. In the lower estuary *H. germanica* calcified in spring or in autumn, whereas *A. tepida* most probably calcified in summer (samples collected in spring 2006) or in summer, autumn or spring (samples collected in winter 2007). Therefore, benthic foraminifera calcified from a few months to half a year (or even more) before sampling. This is particularly true for samples collected in February 2007 in Locmariaquer. In this area benthic foraminifera calcified more than half a year before the collection time. Although the life cycle of benthic foraminifera may be longer than one year (Murray, 1991), their reproduction and growth is limited to a relatively short period, possibly related to favourable environmental conditions. Laboratory experiments demonstrate that *A. tepida* can quickly grow to an adult size in 20 and 120 days depending on temperature, salinity and food availability (Bradshaw, 1957, 1961; Diz et al., in preparation). Observations in our laboratory indicate that after growing to adult size, *A. tepida* can stay alive without further calcification for several months (Diz, unpublished results).

Preferential calcification during warmer months in the lower estuary is probably related to the ecological preferences of the studied species. The lower part of the estuary

is probably not the optimal habitat for *H. germanica* and *A. tepida*, as is shown by their low abundances (Fig. 4b, c). It appears that these taxa only reproduce in the outer estuary during short periods of the year which may coincide with peaks in phytoplankton production in spring and summer (Paulmier, 1972). In the rest of the estuary, the most favourable calcification periods coincide with the colder months and/or early spring. Foraminiferal reproduction peaks in winter and in early spring have been reported in the nearby Bay of Bourgneuf (Morvan et al., 2006).

5.2 Constraints of the approach and future applications

Systematic offsets between the isotopic composition of the foraminiferal shell and the surrounding waters are usually explained as vital effects, which may be the combined effect of multitude of processes (see reviews in Grossman, 1987; Rohling and Cooke, 1999). The consequence is that only few benthic foraminiferal taxa precipitate their shells in equilibrium. To our knowledge, there are no published data available on the isotopic disequilibria for the two species used here; *Ammonia tepida* and *Haynesina germanica*. A small isotopic offset from equilibrium fractionation would shift the lines of the T-S-$\delta^{18}O_{eq}$ plots but would not substantially change the interpretation of our data.

Tidal currents likely transport some live benthic foraminifera from more open marine settings into the estuary and vice versa. For several reasons we consider transport of shells not to have substantially affected the spatial distribution of *H. germanica* and/or *A. tepida*. If foraminifera from the outer parts of the estuary were transported to the upper and middle parts, we should find lighter $\delta^{18}O$ ratios indicative of calcification during the warm season. Additionally, because of their contrasting isotopic signature, we can exclude that specimens collected at Locmariaquer originated from sites in the upper estuary.

Stable isotopic measurements in the Auray estuary represent the average of several individuals which may have calcified in different periods of the year. Isotopic measurements of individual foraminifera would make it possible not only to estimate the calcification period of each single specimen but also to reconstruct the seasonal ranges of

temperature and salinity on the basis of fossil assemblages.

Results obtained in this study show clearly that in subtidal environments, high spatial and temporal variability of the environmental parameters as well as benthic foraminiferal assemblages seriously hamper the application of oxygen and carbon isotope geochemistry for environmental reconstructions. In these extremely variable environments, timing of foraminiferal calcification has a profound impact on the $\delta^{18}O$ of the shells. Here we show that different calcification periods apply to different species, in different parts of the estuary. Although stable isotopes have been used in brackish marine environments to reconstruct paleosalinity over time scales varying from centuries (Thomas et al., 2000; Scheurle and Hebbeln, 2003) to thousands of years (Winn et al., 1998; Kim and Kennett, 1998; Kim et al., 1999; Polyak et al., 2003; Peros et al., 2007), it is obvious that the strong seasonality in these settings needs additional constraints from independent proxies (Polyak et al., 2003; Peros et al., 2007).

6 Conclusions

This study presents a novel approach to interpret stable isotopes in subtidal environments. Foraminiferal $\delta^{18}O$ measurements are compared with theoretical oxygen equilibrium values calculated on the basis of the annual water temperature and salinity cycle in T-S-$\delta^{18}O_{eq}$ diagrams. On the basis of these diagrams the most probable timing of foraminiferal calcification can be determined. Benthic foraminiferal $\delta^{13}C$ is subsequently used to further constrain possible calcification periods. In our study, foraminifera from the upper and lower estuary calcified in different seasons, sometimes several months before they were sampled.

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Table 1. The $\delta^{18}O$ and $\delta^{13}C$ of *A. tepida* and *H. germanica* (>150 µm) from the uppermost centimetre of the sediment (0–1 cm) in the Auray River estuary. Data are reported in ‰ in the Vienna PeeDee Belemnite (VPDB) scale. Replicate samples taken from the same area are indicated by 01 or 02 and duplicate measurements of the same sample by numbers into brackets.

Station	Species	Sampling period	Replicate	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
Kerdaniel	*A. tepida*	6 Apr, 6 May	01	0.985	−2.483
			02	0.641	−2.840
		7 Feb	01	0.005	−2.290
			02(1)	0.243	−2.787
			02(2)	−0.153	−3.206
	H. germanica	6 Apr, 6 May	01	0.447	−3.718
			02	0.159	−2.881
		7 Feb	01	−0.402	−3.121
			02	−0.677	−2.859
Kerdréan	*A. tepida*	6 Apr, 6 May	01	0.565	−2.123
			02	0.067	−2.118
		7 Feb	01(1)	0.517	−2.036
			01(2)	0.618	−2.345
			02	0.410	−2.295
	H. germanica	6 Apr, 6 May	01	0.132	−2.674
			02	0.104	−2.544
		7 Feb	01	0.483	−2.507
Moustoir	*A. tepida*	6 Apr, 6 May	01	0.317	−1.464
			02	0.720	−1.812
		7 Feb	01(1)	1.002	−2.316
			01(2)	0.672	−2.806
			02	−0.412	−2.173
	H. germanica	6 Apr, 6 May	01	0.836	−0.938
			02	0.552	−1.084
		7 Feb	01	−0.112	−1.973
			02	0.395	−1.748
Locmariaquer	*A. tepida*	6 Apr, 6 May	01	−0.585	−0.905
			02	−0.330	0.487
		7 Feb	01	0.042	−0.873
	H. germanica	6 Apr, 6 May	01	0.035	0.004
			02	0.349	−0.782
		7 Feb	01	0.333	−0.524

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Table 2. Calcification periods of *A. tepida* and *H. germanica* in the Auray River estuary. The possible calcification periods are indicated in Fig. 6 by the overlap between the $\delta^{18}O$ foraminiferal values and the areas representing the seasonal $\delta^{18}O_{eq}$ signature. Bold represent the most feasible calcification periods based in the carbon isotopic signature of benthic foraminifera.

Station	Species	Sampling period	Calcification periods	Salinity[a]
Kerdaniel	*A. tepida*	6 Apr, 6 May	Autumn, **winter**	21–22
		7 Feb	Autumn, **winter, early spring**	19–23
	H. germanica	6 Apr, 6 May	Autumn, **winter, early spring**	17–20
		7 Feb	**Late spring, summer**	20–21
Kerdréan	*A. tepida*	6 Apr, 6 May	Autumn, **winter, early spring**	24
		7 Feb	Autumn, **winter, early spring**	23–24
	H. germanica	6 Apr, 6 May	Autumn, **winter, early spring**	21–22
		7 Feb	Autumn, **winter, early spring**	22
Locmariaquer	*A. tepida*	6 Apr, 6 May	**Summer**	28–34
		7 Feb	**Spring, summer, autumn**	29
	H. germanica	6 Apr, 6 May	**Spring, summer, autumn**	33
		7 Feb	**Spring, summer, autumn**	30

[a] Salinity estimates from the carbon isotopic composition of *A. tepida* and *H. germanica*. Estimations are based on the assumption that benthic foraminiferal $\delta^{13}C$ is mainly a function of mixing between sea water and river water (Fig. 3).

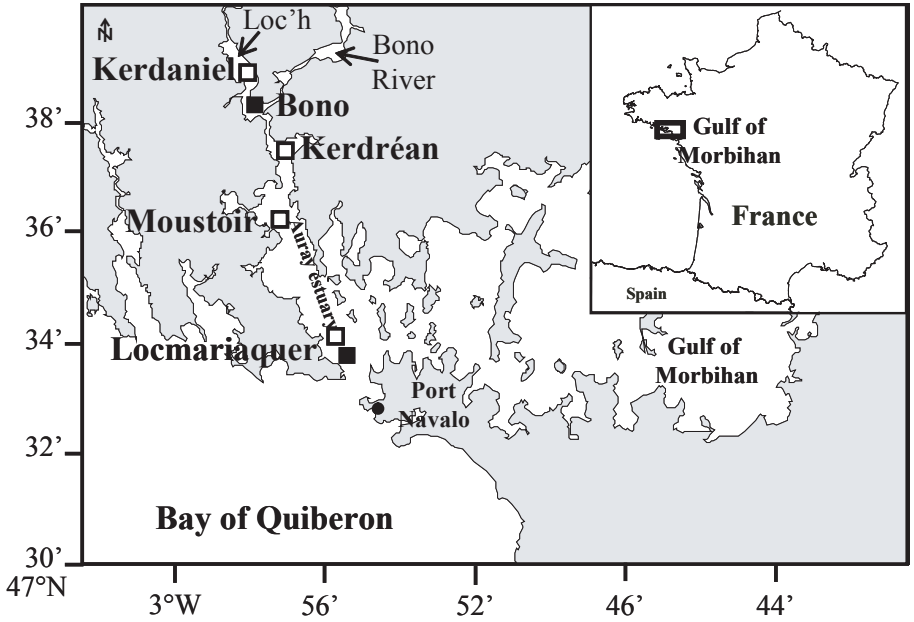

Fig. 1. Setting of the Auray River estuary in the Gulf of Morbihan, foraminiferal sampling sites (white squares) and location of the hydrographic stations (black squares).

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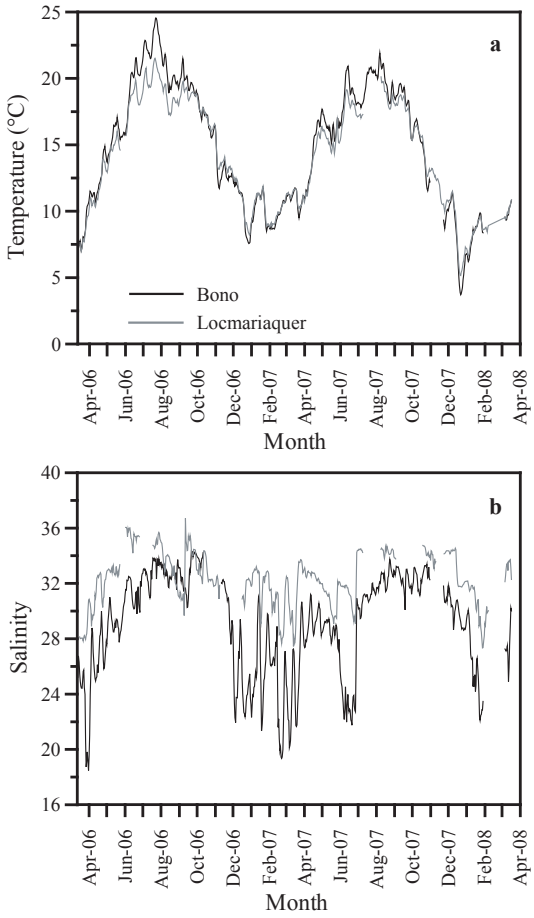

Fig. 2. Bottom water temperature **(a)** and salinity **(b)** in Bono (black line) and Locmariaquer (grey line) between March 2006 and March 2008.

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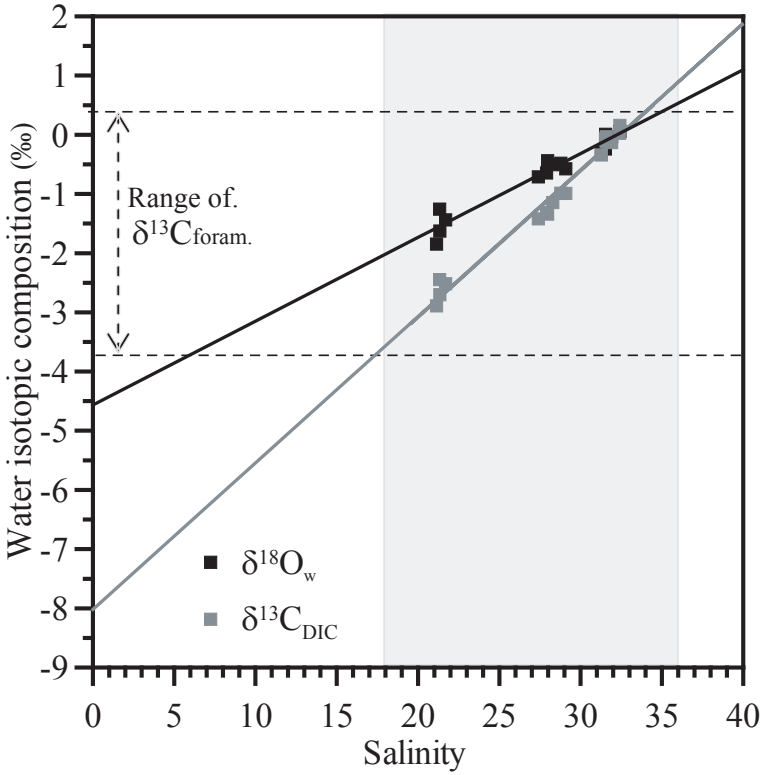

Fig. 3. Mixing line between sea and river waters for $\delta^{18}O_w$ (black) and $\delta^{13}C_{DIC}$ (grey) in the Auray estuary. Oxygen isotopic values (VSMOW, black squares) and carbon isotopic values (grey squares, VPDB) are based on samples collected at Bono (Fig. 1) during 3 tidal cycles. The grey band represents maximum and minimum salinity values measured at Bono and Locmariaquer hydrographic stations (Fig. 2b). Horizontal dashed lines indicate the $\delta^{13}C$ range measured in benthic foraminiferal shells.

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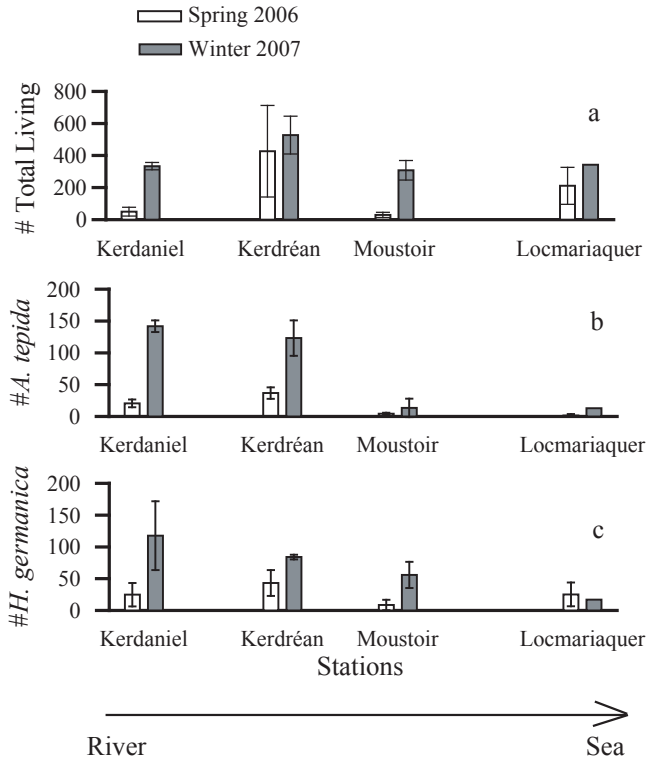

Fig. 4. Mean abundance (white and grey bars) of total living benthic foraminifera **(a)** and of *A. tepida* **(b)** and *H. germanica* **(c)**. The standard deviation (vertical lines) is based on two replicates for each station. White and grey bars refer to spring 2006 and winter 2007, respectively. Vertical axes represents the number of total live individuals (>63 µm) of a particular species found in the core (0–10 cm) and standardised to 50 cm^2 surface area. Note that abundance at Locmariaquer during Winter 2007 does not have a replicate and corresponds to one sample from the uppermost centimetre interval only.

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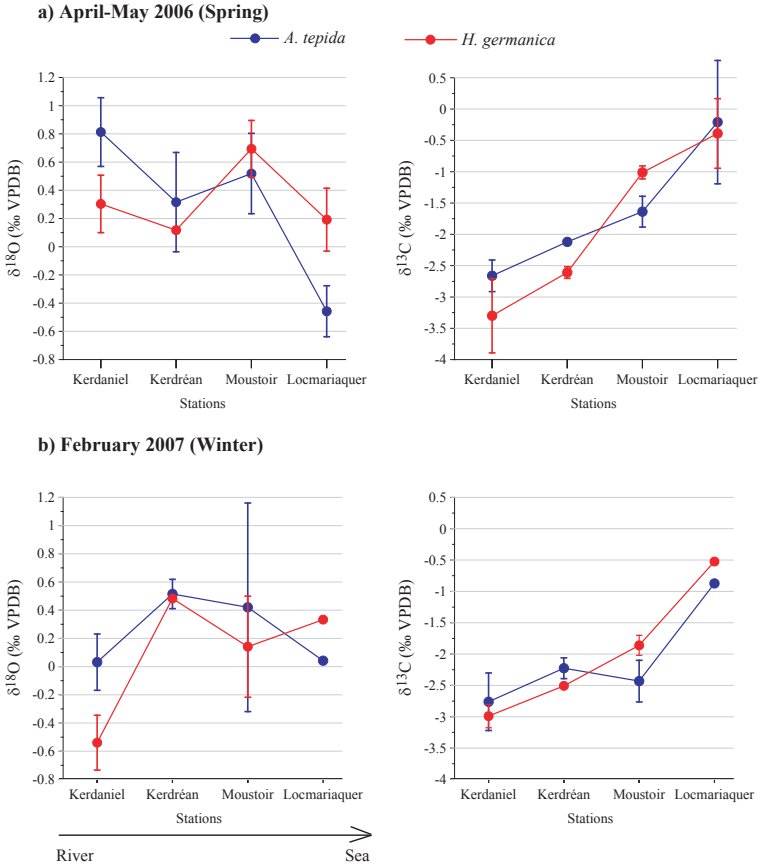

Fig. 5. Carbon and oxygen isotopic composition of *A. tepida* (blue) and *H. germanica* (red) in the Auray River estuary in spring 2006 **(a)** and winter 2007 **(b)** expressed by the mean and the standard deviation. Isotopic measurements are indicated in Table 1.

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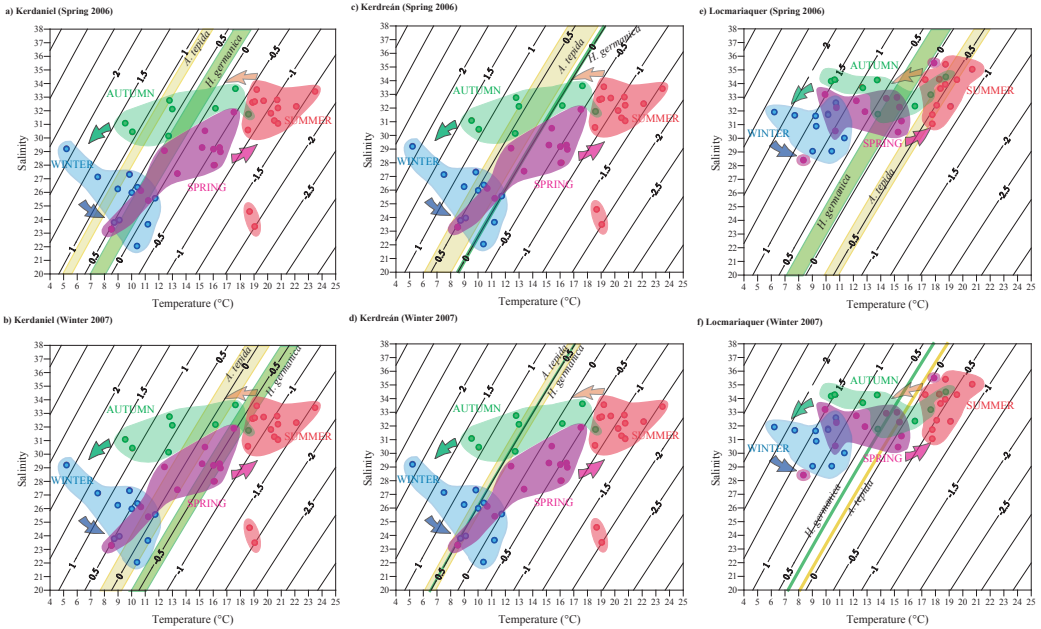

Fig. 6. *T-S-*$\delta^{18}O_{eq}$ diagrams for the Auray River estuary and calcification periods of *A. tepida* and *H. germanica* at Kerdaniel **(a, b)**, Kerdréan **(b, c)** and Locmariaquer **(d, e)**.
The $\delta^{18}O_{eq}$ isolines are calculated according to the paleotemperature equation of Shackleton (1974) and the $\delta^{18}O_w$: salinity relationship for the Auray estuary (see main text). Bottom water temperature and salinity data averaged for 15-day periods are indicated by coloured circles in function of the different seasons. Benthic foraminiferal $\delta^{18}O$ values (Table 1) are plotted in the diagrams as lines, yellow for *A. tepida* and green for *H. germanica*. In case several isotopic measurements have been performed for the same sample a "band" representing the range of measured values is shown. Possible calcification periods are indicated by the overlap of benthic foraminiferal $\delta^{18}O$ values with the annual $\delta^{18}O_{eq}$ cycle.