



HAL
open science

Foraminiferal test dissolution reveals severe sediment acidification in estuarine mudflats: new perspectives for present and historical assessment

Marie Fouet, Maxime Daviray, Emmanuelle Geslin, Edouard Metzger, Frans Jorissen

► To cite this version:

Marie Fouet, Maxime Daviray, Emmanuelle Geslin, Edouard Metzger, Frans Jorissen. Foraminiferal test dissolution reveals severe sediment acidification in estuarine mudflats: new perspectives for present and historical assessment. *Comptes Rendus. Géoscience*, 2024, 356, pp.83-96. 10.5802/crgeos.269 . hal-04634736

HAL Id: hal-04634736

<https://hal.science/hal-04634736>

Submitted on 4 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



ACADÉMIE
DES SCIENCES
INSTITUT DE FRANCE

Comptes Rendus

Géoscience

Sciences de la Planète

Marie Fouet, Maxime Daviray, Emmanuelle Geslin, Edouard Metzger
and Frans Jorissen

**Foraminiferal test dissolution reveals severe sediment acidification in estuarine
mudflats: new perspectives for present and historical assessment**

Volume 1, issue 0 (0000), p. 000-000

<https://doi.org/10.5802/crgeos.269>

 This article is licensed under the
CREATIVE COMMONS ATTRIBUTION 4.0 INTERNATIONAL LICENSE.
<http://creativecommons.org/licenses/by/4.0/>



*The Comptes Rendus. Géoscience — Sciences de la Planète are a member of the
Mersenne Center for open scientific publishing*
www.centre-mersenne.org — e-ISSN : 1778-7025



Research article

Foraminiferal test dissolution reveals severe sediment acidification in estuarine mudflats: new perspectives for present and historical assessment

Marie Fouet^{①, #, a}, Maxime Daviray^{①, #, *, b}, Emmanuelle Geslin^b, Edouard Metzger^{①, b} and Frans Jorissen^{①, b}

^a UMR EPOC, Université de Bordeaux, CNRS, UMR 5805, Station Marine d'Arcachon, 2 rue du Professeur Jolyet, 33120, Arcachon, France

^b Univ Angers, Nantes Université, Le Mans Université, CNRS, LPG UMR 6112, F-49000 Angers, France

E-mails: marie.fouet@u-bordeaux.fr (M. Fouet), maxime.daviray@univ-angers.fr (M. Daviray)

Abstract. Foraminiferal mudflat communities in the Auray estuary, located along the French North-west Atlantic coast, were investigated in September 2020. Numerous living specimens of calcareous species showed marks of dissolution. The number of affected individuals, together with their dissolution degree, varied from one site to another. Moreover, pH profiles showed porewater acidification of the subsurface sediment at several stations. We propose two metrics quantifying the degree of dissolution: (i) the “Foraminiferal Test Dissolution” (FTD), based on binocular observations of the foraminiferal community, and (ii) the “Calcareous Test Preservation Ratio” (CTPR), surface ratio (0–0.5 cm) of total living foraminiferal densities (0–1 cm). In stations showing sediment acidification, the O₂-pH profiles show the typical biogeochemical signatures of cable bacteria activity (pH increase in the oxic zone followed by strong acidification in the suboxic zone). This suggests that bacterial activity could be responsible for the strong pH decrease leading to corrosive porewaters affecting calcareous tests, hence severe FTD in the first centimetre of sediment leading to high CTPR. Furthermore, observations of FTD in other estuaries on the French Atlantic coast indicate that the phenomenon has become widespread. Re-investigated foraminiferal tests sampled in 1995–1996 in the Auray estuary show no dissolution marks, suggesting that the dissolution process observed in 2020 appeared over the last thirty years. Therefore, the FTD and CTPR could be used as rapid tools to establish the occurrence of dissolution processes, pending geochemical confirmation. The FTD could also be used as sediment acidification proxy in historical records, where geochemical analyses are not possible.

Keywords. Benthic foraminifera, Test preservation, Cable bacteria, Sediment acidification proxy.

Funding. CNRS-INSU (program LEFE-CYBER, project CB-FOR), Angers University, OFB (French Office of Biodiversity, grant number 3976-CT-RD-AMI-18-SURV-FORESTAT).

Manuscript received 21 March 2024, revised 4 June 2024, accepted 24 June 2024.

* Corresponding author.

Contributed equally.

1. Introduction

Estuaries are complex, dynamic and very productive areas [Day *et al.*, 2012]. Despite their low diversity, estuarine habitats host high abundances of macro- and meiofauna, because they provide shelter [Elliott and McLusky, 2002] and are storehouses of nutrients and organic matter [Elliott and Quintino, 2007]. Organisms living in estuarine intertidal mudflats must endure intense environmental stress, as a result of daily (tidal cycles) to monthly (precipitation and river runoff) changes in marine and freshwater inputs [Elliott and McLusky, 2002, Jorissen *et al.*, 2022]. Besides those stresses, coastal environments are increasingly threatened by ocean acidification [Cai *et al.*, 2021, Cooley *et al.*, 2022, Wootton *et al.*, 2008]. This phenomenon jeopardises benthic organisms producing calcareous shells such as foraminifera.

Benthic foraminifera are widely present in estuarine areas [Debenay *et al.*, 2000b]. These unicellular protists have the particularity to build a shell (called test), using different materials for its construction. Some species produce an organic test, others aggregate sediment particles, while a large group of species produce a test of calcium carbonate [de Nooijer *et al.*, 2009, Debenay *et al.*, 2000a]. Numerous authors have studied the distribution of foraminifera in estuarine mudflats [e.g., Moreno *et al.*, 2005, Debenay *et al.*, 2006, Mojtahid *et al.*, 2016, Francescangeli *et al.*, 2020, Thibault de Chanvalon *et al.*, 2022]. The benthic foraminifera communities in the Auray estuary (French Atlantic coast) were investigated 30 years ago by Redois [1996] and recently in 2019 by Fouet *et al.* [2022] and 2020 by Daviray *et al.* [2024]. In 1996, Redois did not mention any marks of dissolution of calcareous foraminifera, whereas Fouet *et al.* [2022] and Daviray *et al.* [2024] noted at several stations numerous specimens of calcareous foraminifera with partly or completely dissolved test.

Numerous authors have described dissolution of calcareous tests of living foraminifera in coastal environments and various hypotheses have been put forward to explain the causes of the dissolution phenomenon. For instance, abiotic factors such as trace metal pollution [Buzas-Stephens *et al.*, 2018], re-oxidation of reduced dissolved or particulate sulphidic compounds [Cesbron *et al.*, 2016] or freshwa-

ter intrusion [Charrieau *et al.*, 2018, Haynert *et al.*, 2012] have been suggested to reduce the pH of pore water. Biotic factors which can reduce the saturation state of calcite have also been proposed such as intense microbial respiration of organic matter [Schönfeld and Mendes, 2022] or cable bacteria activity [Daviray *et al.*, 2024].

Cable bacteria (CB) were discovered a decade ago in coastal marine sediments [Nielsen *et al.*, 2010, Pfeffer *et al.*, 2012]. Their activity (CBA) produces an electric gradient leading to ion migrations and a very particular geochemical signature in the sediment: a pH maximum in the oxic zone just below the sediment surface, followed by a strong pH decrease in the few centimetres below [Meysman *et al.*, 2015, Nielsen *et al.*, 2010, Pfeffer *et al.*, 2012, Risgaard-Petersen *et al.*, 2015, 2012]. CB have been described in various aquatic environments (e.g. open sea [e.g. Baltic Sea, Hermans *et al.*, 2019; Black Sea, Hermans *et al.*, 2020], marine lakes [e.g. Grevelingen, Malkin *et al.*, 2014], intertidal salt marshes [e.g. New England, Larsen *et al.*, 2014], sandy intertidal flats [e.g. Wadden Sea, Malkin *et al.*, 2017] and also in freshwater soils [e.g. Giber Å, Denmark, Risgaard-Petersen *et al.*, 2015]), all around the world, see Burdorf *et al.* [2017] for a more extensive review of CBA occurrence worldwide. In French marine environments, CB were also observed especially in Mediterranean Lagoons such as Berre [Dam *et al.*, 2021], Thau [Burdorf *et al.*, 2017] in the Gulf of Lion and Urbino in Corsica [Burdorf *et al.*, 2017]. Cable Bacteria were also observed in French Atlantic intertidal mudflats such as Arcachon bay and Auray estuary [Daviray *et al.*, 2024].

Here, the new dataset of living foraminiferal communities sampled in 2020 in the Auray estuary is accompanied by oxygen and pH microprofiling to better understand the biogeochemical processes behind the dissolution of foraminiferal tests. To better constrain the significance of these observations, the samples from the same area collected in 1995–1996 [Redois, 1996] and in 2019 [Fouet *et al.*, 2022] were re-examined. In addition, we re-investigated samples from other estuaries located along the French Atlantic coast [Fouet, 2022]. This short article signals the emergence of CBA in the sediments along the French Atlantic coast, and the repercussions in terms of environmental chemistry, ecology, and taphonomic processes.

2. Material and methods

The Auray estuary (Brittany, France; Figure 1) is a typical ria (drowned river valley), connected to the Morbihan Gulf (an enclosed marine bay), located along the north French Atlantic coast. This zone is subjected to a mesotidal to low macrotidal regime with a tidal range of about 4 m. More details about the studied site can be found in Fouet *et al.* [2022]. Living foraminiferal samples were collected in September 2020. All 10 stations were sampled along the estuary at low tide (Figure 1B). Stations 6B, 4B, 2C of this study corresponds to stations 1, 2, 3 respectively in Daviray *et al.* [2024], who also investigated the presence of CB by qPCR at stations 1 (6B) and 2 (4B).

At each station, the first two 0.5 cm of the sediment were collected by hand using tubes with an internal diameter of 9.6 cm. Samples were stained with 2 g/L Rose Bengal and preserved in 96% ethanol. In the laboratory, samples were sieved over a 125 μm mesh. All stained foraminifera were picked in water, using a Leica MZ16 stereomicroscope. Based on stereomicroscope observations of calcareous tests contained in each sample (examples of individuals showing dissolution marks are shown in Figure 2), three types of samples were distinguished, characterised by different type of Foraminiferal Test Dissolution (FTD):

- Type 1 (no visible test dissolution)—Most individuals have a transparent, glassy test; less than 10% of the test is whitish and opaque; this is considered as the first sign of dissolution.
- Type 2 (moderate dissolution)—More than 10% of the individuals have a whitish, matt test, while some specimens show more advanced stages of test dissolution.
- Type 3 (advanced to severe test dissolution)—Most specimens show holes and cracks in the test with organic lining sometimes becoming partially or completely visible. Some glassy specimens may still be present in the assemblage.

The 0–0.5 and 0.5–1 cm levels were studied separately, and the overall assessment of FTD type of each sample was based on the layer which showed the most severe dissolution (nearly always the 0.5–1 cm level). To investigate whether dissolution caused the loss of foraminiferal tests in slightly deeper sediment

layers, we calculated a Calcareous Test Preservation Ratio (CTPR) that was defined as the ratio between the density of tests of living calcareous foraminifera in the 0–0.5 cm level and their total density in the 0–1 cm layer. When CTPR was below 0.6, high densities of calcareous specimens were recorded in the subsurface layer (0.5–1 cm); when CTPR was between 0.6 and 0.90, their densities were higher in the surface layer, and when CTPR was above 0.90, the population living in the subsurface layer had largely disappeared.

To investigate both the spatial and historical extent of dissolution processes, additional samples were re-examined using a stereomicroscope to define the FTD. Concerning the spatial extent, samples from Fouet [2022] were used to describe the FTD of samples coming from eight other estuaries (133 samples) (Figure 1A). Concerning the historical aspect, Auray estuary samples collected 25 years ago [Redois, 1996] and in 2019 [Fouet *et al.*, 2022] were also inspected for FTD and CTPR values. Samples collected by Fouet and co-workers in 2019 used the same methodology as presented here. However, their study performed triplicates. The methodology used by Redois [1996] was slightly different. He studied the whole 0–1 cm level, collected with a spoon on a surface of 100 cm³, stained with Rose Bengal and preserved in ethanol, washed on a 50 μm mesh size sieve and dried the samples for binocular observation. Therefore, CTPR could not be calculated for 1995–1996 samples, and these samples were only investigated for FTD.

Oxygen and pH microprofiling were performed at each station. After retrieval in the field, cores were immersed in an aquarium with estuarine water at *in situ* temperature, where oxygen saturation was maintained by air-bubbling. Two Unisense© profiling systems were used simultaneously. One consisted of two Clark-type oxygen microsensors with a 50 μm tip [Revsbech, 1989, Revsbech and Jørgensen, 1986], and the other of a pH sensor with a 500 μm tip diameter (PH500, Unisense©). Both microsensors were mounted on a motorised micromanipulator linked to a computer, and connected to a MultiMeter S/N. We used a 50- μm increment for oxygen, whilst for pH we chose a 100- μm increment around the sediment-water interface, which was further adapted in real-time according to the evolution of the observed pH profile, down to 4–5 cm depth. To calibrate the oxygen microsensor, two points were used, the water

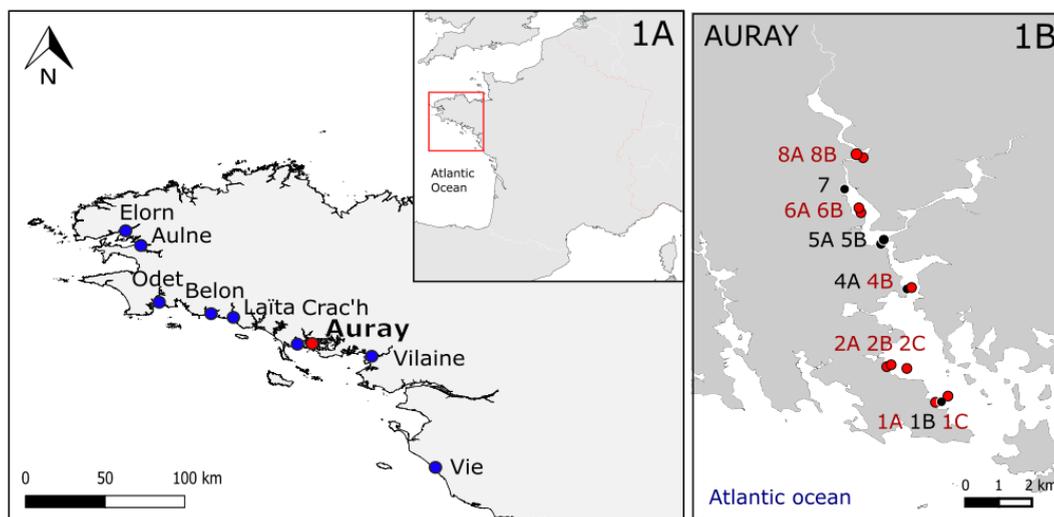


Figure 1. 1A: Location of the studied estuaries (in red: Auray estuary, in blue: eight other estuaries). 1B: Location of the stations in the Auray estuary sampled in 2019 and 2020 (oxygen and pH profiles were measured in 2020 for stations indicated in red). Modified from Fouet [2022].

column for 100% saturation and pore waters in the anoxic sediment for 0%. For pH calibration, three NBS buffers were used (values 4.0, 7.0 and 9.2).

3. Results and discussion

3.1. Sediment geochemistry

Figure 3 shows oxygen and pH profiles at and below the Sediment-Water Interface (SWI) for 10 stations in the Auray estuary taken in September 2020. All stations showed typical pore-water oxygen profiles with a strong decrease in the topmost sediment. The oxygen penetration depth (OPD) ranged from 0.7 to 1.5 mm depth. Based on the pH profiles, which showed greater variability, three groups of stations could be distinguished:

- Group I (stations 2A, 6A, 8A, 8B): the pH strongly decreased from ~ 7.8 at the SWI to ~ 7.0 at 2–4 mm depth, to remain relatively stable in deeper sediment layers.
- Group II (stations 1C and 6B): the stations showed pH profiles with slightly acidic pore waters in the suboxic zone. Station 1C showed a minor pH peak (7.9) just below the SWI, but deeper down, the pH remained stable around 6.6 until 4 cm depth. Station 6B

showed a minor acidification in the suboxic zone (pH = 6.8). According to Daviray *et al.* [2024], DNA sequencing analysis at station 6B (station 1 in their article) showed low cable bacteria densities.

- Group III (stations 1A, 2B, 2C, 4B): the pH decreased to a minimum well below 7.0 between 0.5 and 1.0 cm depth, in the suboxic zone (i.e., without detectable oxygen). It increased again towards greater depth but remained below 7.0. In addition, the profiles of stations 2C and 4B showed a small but distinct pH peak just below the SWI. According to Daviray *et al.* [2024], DNA sequencing analysis at station 4B (station 2 in their article) showed high CB densities.

O₂-pH profile groups can be discriminated according to the knowledge of early diagenesis processes and geochemical fingerprint of cable bacteria activity [CBA; Meysman *et al.*, 2015, Nielsen *et al.*, 2010, Pfeffer *et al.*, 2012, Risgaard-Petersen *et al.*, 2012, 2015]. Profiles of group I are considered as typical for marine environments without CBA. Conversely, the profiles of group III suggest acidification stems from CBA as shown in Nielsen *et al.* [2010]; Pfeffer *et al.* [2012] and Meysman *et al.* [2015]. Daviray *et al.* [2024] confirmed the presence of CB [*Candidatus* Electrothrix

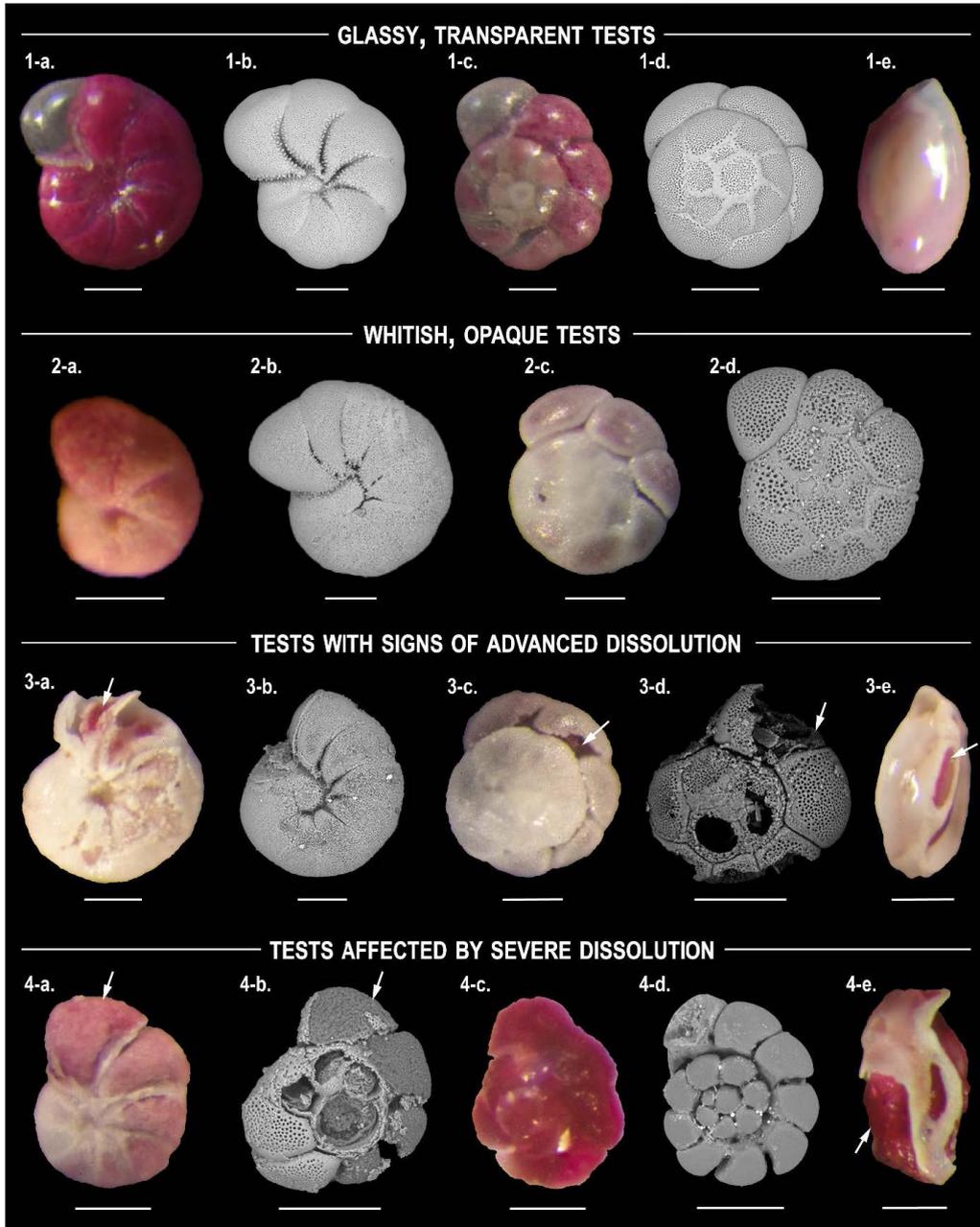


Figure 2. Examples of gradual foraminiferal test dissolution. (1) Glassy, transparent tests, (2) Whitish, matt tests, (3) Tests with signs of advanced dissolution, (4) Tests affected by severe dissolution, for *Haynesina germanica* (1-a, 1-b, 2-a, 2-b, 3-a, 3-b, 4-a), *Ammonia* spp. (1-c, 1-d, 2-c, 2-d, 3-c, 3-d, 4-b, 4-c, 4-d) and *Quinqueloculina oblonga* (1-e, 3-e, 4-e). For each dissolution stage, figures a, c and e have been produced with an optical microscope, figures b and d with a Scanning Electronic Microscope. White arrows indicate the organic lining; scale bars: 100 μm .

sp.; Trojan *et al.*, 2016] at station 4B by DNA analysis and estimated a CB filament density of $74.4 \pm$

$5.0 \text{ m}\cdot\text{cm}^{-3}$ per bulk sediment in the first sediment centimetre. Finally, in group II, O_2 -pH profiles show

only a slight pH decrease and are not typical of intense CBA. Even if the pH profile do not show a clear pH decrease, Daviray *et al.* [2024] indicated the presence of genetical material of cable bacteria in station 6B in 2020 with a low CB filament density of $7.4 \pm 0.4 \text{ m}\cdot\text{cm}^{-3}$ per bulk sediment. Therefore, these two profiles are interpreted as sustaining moderate CBA.

3.2. Foraminiferal assemblages

The density of living foraminifera (excluding organic linings) in the first centimetre varied from 76 to 5120 individuals per 50 cm^3 with 52–2164 individuals per 50 cm^3 in the surface half centimetre and 22–3575 individuals per 50 cm^3 in the subsurface (0.5–1 cm) layer (Figure 4). These values are comparable to those observed in 2019 [Fouet *et al.*, 2022] and in other estuaries [e.g., Camacho *et al.*, 2015, Debenay *et al.*, 2006, Francescangeli *et al.*, 2021, Mojtahid *et al.*, 2016]. The foraminiferal density was generally higher in the superficial sediment layer (0–0.5 cm) than in the 0.5–1 cm layer, except for station 8B. For many stations (i.e., 1A, 4B), the densities were an order of magnitude lower in the second layer.

The Calcareous Test Preservation Ratio (CTPR), which describes the density differences within the first centimetre, varied between 0.27 and 0.96 (Table 1). Five stations showed values of CTPR higher than 0.6 (1A, 1C, 2B, 2C and 4B) suggesting a low occurrence of calcareous living foraminifera in the subsurface layer (0.5–1 cm). At stations of acidification groups II and III, dissolution, as shown by FTD was much more intense in the 0.5–1 than in the 0–0.5 cm layer, and consequently, foraminiferal densities were much higher in the first layer (Figure 4), leading to a high CTPR (Table 1). This is not surprising in view of the pH profiles of group III (Figure 3), which show that the strong pH minimum ($5.3 < \text{pH} < 6.3$) was always positioned between 0.5 and 1.0 cm depth.

Table 1 shows the comparison of the results of the pH profiles, Foraminiferal Test Dissolution (FTD) and the Calcareous Test Preservation Ratio (CTPR). In many cases, scores yielded by these three methods converge. All three indicators suggest that there was intense sediment acidification at stations 1A and 4B, and to a lesser degree at stations 2B and 2C. At these stations, FTD was maximal, especially in the 0.5–1 cm layer, leading to high CTPR values (>0.6). Conversely,

at stations where the pH profile did not indicate sediment acidification (i.e., 2A, 6A, 8A and 8B), the observed FTD was minimal and the CTPR was always below 0.6, indicating that calcareous individuals were well preserved in both sediment levels. Stations of pH group II (with atypical pH profiles) showed intermediate FTD and CTPR values, confirming our initial interpretation that these stations were slightly affected by acidification. However, there are some exceptions to this pattern. At stations 2A and 8B, where the pH profile suggested no acidification, moderate FTD was observed. Nevertheless, it appears that in the case of pH profiles indicative of strong acidification, the living foraminiferal assemblages show advanced to severe FTD, which is confirmed by an important loss of calcareous tests in the 0.5–1.0 cm layer, leading to a high CTPR.

These results show a good correspondence between observations of foraminiferal test dissolution and measurements of sediment acidification. In the literature, different causes of foraminiferal test dissolution have been proposed. First, global ocean acidification is one of the threats that could alter organisms that produce calcareous shells such as foraminifera [Guamán-Guevara *et al.*, 2019, Kuroyanagi *et al.*, 2021]. However, these changes are expected to impact all sites of the same ecosystem equally, so this explanation was not retained to explain the differences in sediment acidification observed between the different stations of this study. Therefore, the possibility of freshwater intrusion to explain the FTD [Charrieau *et al.*, 2018, Haynert *et al.*, 2012] is not retained either, because stations located in the inner part, and receiving more freshwater input (stations 8A and 8B), do not show stronger acidification than stations located in the outer part of the estuary (1A and 1C). Buzas-Stephens *et al.* [2018] proposed that trace metal pollution can explain FTD, but in our stations, measurement of trace metal did not show any sign of major pollution [Fouet *et al.*, 2022].

Based on the similarities between our pH profiles showing sediment acidification associated with oxygen profiles (group III) and the representative one of Cable Bacteria Activity, as presented in Meysman *et al.* [2015], Nielsen *et al.* [2010] and Pfeffer *et al.* [2012], we hypothesised that CBA could be responsible of Foraminiferal Test Dissolution at these Auray estuary stations (1A, 2B, 2C, 4B). However, some

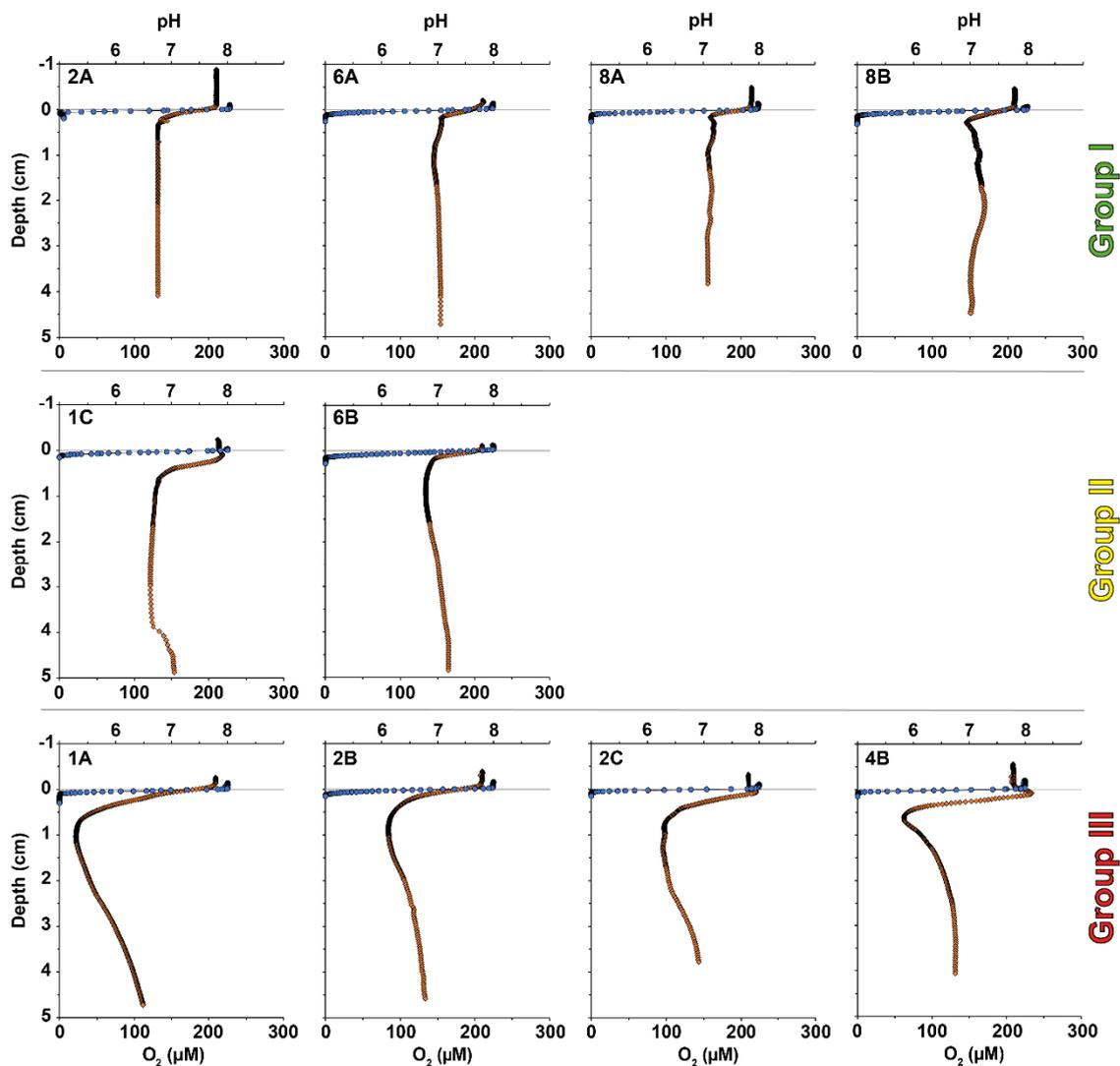


Figure 3. pH (orange) and oxygen (blue) profiles measured in September 2020 in the Auray estuary. Three groups were established according to the shape of the O₂-pH profiles (group I: low acidification; group II: moderate acidification; group III: strong acidification). Profiles from stations 2C, 4B and 6B are also published in Daviray *et al.* [2024].

discrepancies between the pH profiles and the observations of FTD and CTPR could be noted, for which two explanations can be advanced. First, as suggested by differences in FTD between stations collected in the same mudflat (i.e., station 1A versus 1C, and station 2A versus 2C and 2B or station 6A versus 6B), CBA could have a patchy distribution on the mudflat. The pH measurements are based on a few 1D punctual profiles (500 μm scale) across the depth of the sediment, whereas foraminiferal observations

included a large sediment volume (72 cm^3). The foraminiferal sampling zone may have been subject to scattered bacterial activity. Next, while pH measurements suggest acidification at the moment of sampling, the living foraminiferal community could reflect dissolution that has occurred over the last few weeks [Charrieau *et al.*, 2018, 2022, Le Cadre *et al.*, 2003]. Indeed, several authors have shown that CBA may vary considerably throughout the year [Seitaj *et al.*, 2015, Sulu-Gambari *et al.*, 2016].

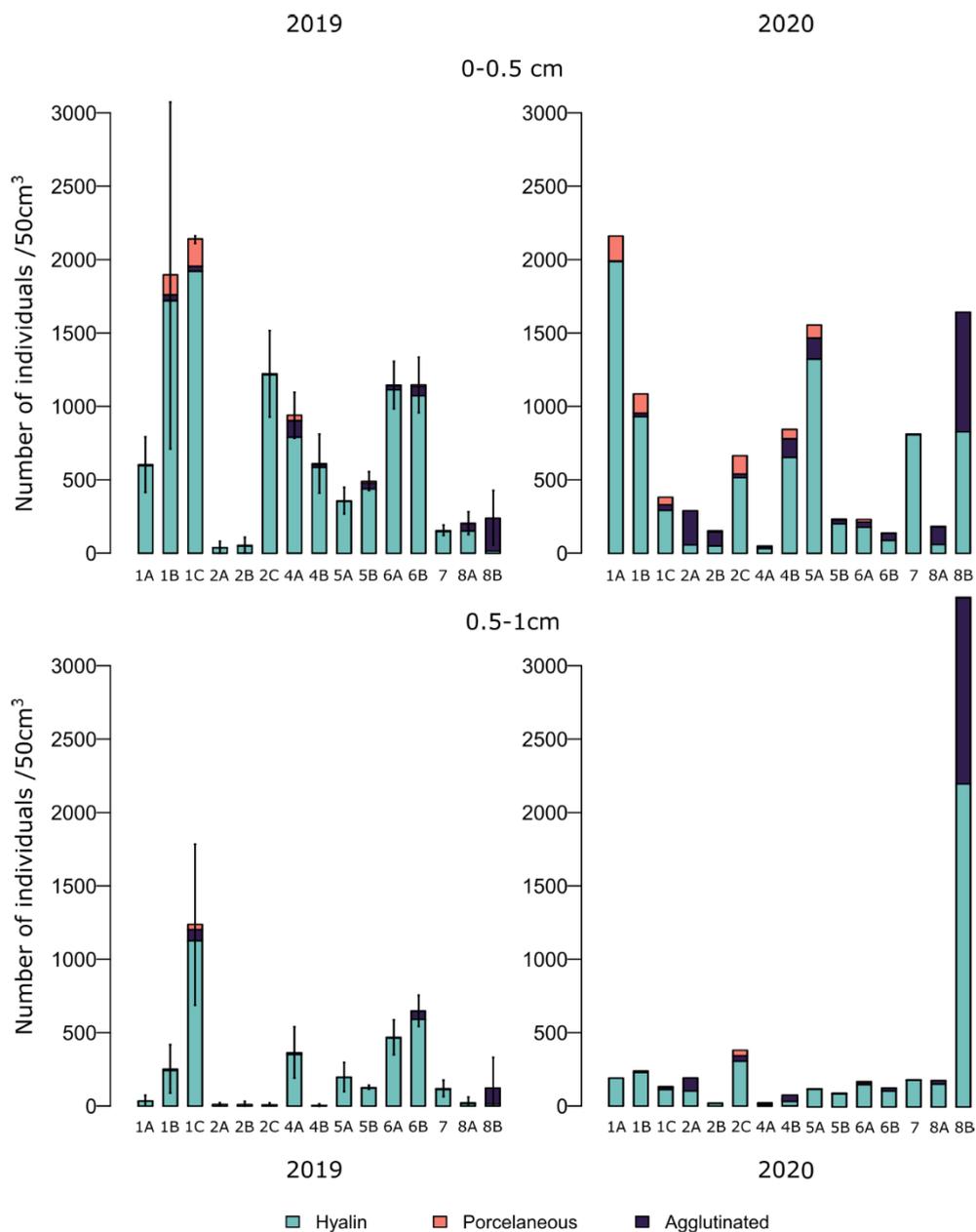


Figure 4. Foraminiferal density in the $>125 \mu\text{m}$ fraction for the 0–0.5 and 0.5–1 cm levels, expressed in individuals per 50 cm^3 (for samples collected in July 2019 by Fouet *et al.* [2022] and September 2020 (this study)). The three replicates of 2019 were averaged, and the standard deviation is presented overhead. The stations are ordered from the outer (left) to the inner part of the estuary (right).

In order to explore whether FTD also occurs on individuals collected in other estuaries, samples from eight other estuaries [Figure 1; Fouet, 2022] were visually inspected for marks of dissolution. The results

of these observations are presented in Table 2, using the same three types of sample classification, characterised by different stages of FTD, as in the Aurray estuary. A few stations from Odet, Laita and

Table 1. The first row indicates the O₂-pH profile groups from green (group I: low acidification) through yellow (group II: moderate acidification) to red (group III: strong acidification). The second row describes the CTPR, with <0.6: green, 0.6–0.90: yellow, >0.90: red. The last row summarises observations of foraminiferal test dissolution (FTD), ranging from “no visible dissolution” (type 1, green), via moderate (type 2, yellow) to “advanced to severe dissolution” (type 3, red)

Auray station (2020)	1A	1C	2A	2B	2C	4B	6A	6B	8A	8B
Acidification intensity	III	II	I	III	III	III	I	II	I	I
CTPR (calcareous test preservation ratio)	0.91	0.72	0.36	0.73	0.63	0.96	0.54	0.45	0.28	0.27
FTD (foraminiferal test dissolution)	3	2	2	3	3	3	1	2	1	2

Crac’h estuaries showed moderate marks of test dissolution. Both Aulne and Belon estuaries had one station showing advanced to severe marks of test dissolution (Table 2). By analogy with Auray, we hypothesise that CBA could be present in these estuaries as well. This still remains to be confirmed by more distinctive methods such as pH profiling or molecular identification. Conversely, no FTD was observed in the Vie (two different field campaigns, in October 2018 and June 2020), Elorn and Vilaine estuaries. Also in the Loire estuary, re-inspection of foraminiferal assemblages described by Mojtahid *et al.* [2016] did not show any signs of FTD suggesting the absence of foraminiferal test dissolution at these locations (com. pers. Mojtahid). Moreover, concerning the geographical extent of this strong acidification phenomenon, advanced to severe test dissolution of living foraminifera have earlier been described in Arcahion Bay [Cesbron *et al.*, 2016]. The authors explained this as the result of decreased pH due to bacterial activity around decomposing eelgrass roots. However, closer inspection of their pH profiles suggests a CBA signature. Together with the strong decrease of calcareous tests densities in the first centimetre of the sediment could also plead to intense seasonal cable bacteria activity. Summarising, in several intertidal mudflats of French transitional environments (estuaries and semi-enclosed bays), FTD has been observed that could be the result of CBA. It therefore appears that this acidification phenomenon could be widespread along the French coast.

In addition, to look into the historical aspect of the presence of this phenomenon, we re-examined two foraminiferal datasets from Auray estuary: samples

with three replicates per station collected in July 2019 [Fouet *et al.*, 2022] and samples collected during a 10-month survey in 1995–1996 [Redois, 1996]. Samples were collected on the same mudflat as the present study (see method section). Concerning the samples collected in 2019 (Table 3), advanced to severe test dissolutions (FTD type 3) was observed at several stations and in several replicates that corresponds to similar FTD type found in the present study. It can be noted that CTPR values from calcareous foraminiferal communities collected in 2019 (Table 3) were in good agreement with those observed in 2020 for stations 1A, 1C, 2B, 4A, 5B, 7. CTPR values (Table 3) for the stations 2A, 2C, 6B and 8A show higher values in 2019 than in 2020 (>0.9 on average) while station 5A shows a higher CTPR value higher in 2020 (>0.95). Differences between samples could be attributed either to interannual variability (between 2019 and 2020) or to spatial heterogeneity (between 2019 replicates) of CBA. Concerning the samples collected in 1995–1996, although the sampling methodology and treatment were slightly different (0–1 cm layer sampled and samples dried so that organic linings were not preserved), visual inspection of the calcareous foraminiferal tests was still possible. The re-examination of the foraminiferal tests of these samples did not yield any marks of foraminiferal test dissolution, and all examined samples were classified as Type 1. This suggests that in the Auray estuary, sediment acidification is a recent phenomenon (present at least since 2019) that appeared in this area in the last 25 years.

To better assess the spatial and temporal dynamics of this phenomenon, the re-examination of

Table 2. Values of FTD (Foraminiferal Test Dissolution)

Auray (2019)			Elorn (2020)	Aulne (2020)	Odet (2020)	Belon (2019)	Laïta (2020)	Crac'h (2020)	Vilaine (2019)	Vie (2018)			Vie (2020)										
a	b	c								a	b	c											
1A	3	3	3	1	1	1	3	1	2	1	1	1	1	1	1	2	1						
1B	2	2	2	2	1	2	1	2	1	3A	1	2	1	2	2	1B	1	2	1	1	1	3	1
1C	2	2	2	3A	1	3	1	3	1	3B	1	3	2	3	1	1C	1	3	1	1	1	4	1
2A	3	3	3	3B	1			4	2	4A	1			4	1	2A	1	4	1	1	1	5	1
2B	3	3	3	4	1					4B	1					2B	1	5	1	1	1	7A	1
2C	3	3	3							5A	1					3	1	6	1	1	1	7B	1
4A	1	3	1							5B	1							7A	1	1	1	7C	1
4B	3	3	3							6A	3							7B	1	1	1	8A	1
5A	1	2	1							6B	1							7C	1	1	1	8B	1
5B	2	2	2															8A	1	1	1	9	1
6A	1	1	1															8B	1	1	1	10A	1
6B	2	2	2															9	1	1	1	10B	1
7	2	2	2															10A	1	1	1		
8A	3	3	1															10B	1	1	1		
8B	-	-	-																				

Stereomicroscope observations of foraminiferal test dissolution, ranging from “no visible test dissolution” (group 1, green) to “moderate” (group 2, yellow), “advanced to severe dissolution” (group 3, red) for samples collected in nine different estuaries studied in Fouet [2022]. The letters a, b, and c are the three replicates sampled in 2018 (Vie estuary) and 2019 (Auray estuary).

Table 3. Values of CTPR: ratio between the number of calcareous individuals in the 0–0.5 cm level and the total number of calcareous foraminifera in the 0–1 cm sample, for the three replicates (a, b, c) sampled in 2019 [after Fouet *et al.*, 2022] and 2020 at all the fifteen stations along the Auray estuary

Station		1A	1B	1C	2A	2B	2C	4A	4B	5A	5B	6A	6B	7	8A	8B	
CTPR (calcareous test preservation ratio)	2019	a	0.98	0.91	0.55	0.80	0.81	0.99	0.54	1.00	0.73	0.83	0.69	0.66	0.77	0.82	-
		b	0.99	0.88	0.80	0.71	0.81	1.00	0.82	1.00	0.74	0.75	0.79	0.61	0.52	1.00	-
		c	0.87	0.88	0.59	0.84	1.00	0.99	0.72	1.00	0.47	0.75	0.63	0.66	0.42	1.00	-
	2020		0.91	0.80	0.72	0.36	0.73	0.63	0.73	0.96	0.92	0.69	0.54	0.45	0.81	0.28	0.27

“-” indicates the sample where the density of calcareous species is <10 specimens.

foraminiferal tests collected in earlier studies is possible. Observation of archived samples may show FTD indicative of former acidic conditions at places where their presence had not been suspected in the past. This method represents a first, rapid and inexpensive approach for directing further analyses in order to characterise present and past dissolution processes using other approaches (elemental and isotopic analyses, *sedDNA*, etc.). We therefore recom-

mend for future investigations studying foraminiferal living assemblages to use a resolution of 0.5 cm in the uppermost sediment to allow CTPR calculation. In addition, the ideal condition for observing FTD is a sediment that has been kept humid, in which foraminifera have been coloured (i.e., Rose Bengal or CTG) allowing inner organic linings observation. Thereby, the recognition of FTD in fossil assemblages preserved in sediment archives could

yield information about the historical evolution of CBA, as shown by Richirt *et al.* [2022]. These authors observed in a sediment core from Grevelingen Lake (The Netherlands) very low densities of calcareous foraminifera at 20 cm depth, corresponding to a period of major change, the reoxygenation of bottom water due to the increase of water exchanges after the opening of the lake to the sea. They hypothesise that such changes could have promoted the enhancement of CBA since 2003, and therefore carbonate test dissolution. Such investigations could help to better understand the spread of these recently discovered bacteria, remaining poorly known, and their consequences for the ecology of the surrounding organisms. Species with a calcareous test could be more fragile, and others (such as agglutinated foraminifera or soft-walled organisms) more adapted to resist to dissolution, so this additional stress could lead to changes in the community structure [Daviray *et al.*, 2024]. In addition, sediment acidification can result in a loss of sediment archives [Daviray *et al.*, 2024], which could have implications for the interpretation of palaeontological data.

4. Conclusion

The foraminifera from sediment samples collected in September 2020 in the Auray estuary showed severe marks of dissolution. This process was not homogenous along this estuary. In addition, pH profiles showed acidification of the porewater at a few millimetres depth after the oxic zone in several stations. These profiles are typical of cable bacteria activity signature. We suggest that an important loss of calcareous foraminifera in the first half centimetre (high CTPR) coupled with foraminiferal test dissolution (FTD) can be considered as an indicator of a potential occurrence of porewater acidification, such as cable bacteria activity. It appears therefore that these metrics could be used as a rapid tool of sediment acidification, pending geochemical confirmation. FTD could also be used as a proxy of acidic conditions in historical records, where pH can no longer be measured. The study of FTD in samples collected at the Auray estuary in the 90s suggests that these acidic conditions have emerged over the last thirty years. Re-examination of calcareous foraminifera coming from other estuaries on the French Atlantic

coast allows to propose that the corrosive phenomenon is or has become widespread over the last few decades.

Declaration of interests

The authors do not work for, advise, own shares in, or receive funds from any organization that could benefit from this article, and have declared no affiliations other than their research organizations.

Funding

The authors received funding from the CNRS-INSU (program LEFE-CYBER, project CB-FOR), from the Angers University and the OFB (French Office of Biodiversity, grant number 3976-CT-RD-AMI-18-SURV-FORESTAT).

Acknowledgements

The authors are grateful to Nils Risgaard-Petersen for his advice during the early stages of the writing process, and Robin Fentimen for his proofreading and correction language of the text. The authors thank the participants in the field trips (2019 and 2020).

References

- Burdorf, L. D. W., Trammer, A., Seitaj, D., Meire, L., Hidalgo-Martinez, S., Zetsche, E.-M., Boschker, H. T. S., and Meysman, F. J. R. (2017). Long-distance electron transport occurs globally in marine sediments. *Biogeosciences*, 14, 683–701.
- Buzas-Stephens, P., Buzas, M. A., Price, J. D., and Courtney, C. H. (2018). Benthic Superheroes: living foraminifera from three bays in the Mission-Aransas National Estuarine Research Reserve, USA. *Estuaries Coasts*, 41, 2368–2377.
- Cai, W.-J., Feely, R. A., Testa, J. M., Li, M., Evans, W., Alin, S. R., Xu, Y.-Y., Pelletier, G., Ahmed, A., Greeley, D. J., Newton, J. A., and Bednaršek, N. (2021). Natural and anthropogenic drivers of acidification in large estuaries. *Annu. Rev. Mar. Sci.*, 13, 23–55.
- Camacho, S., Moura, D., Connor, S., Scott, D., and Boski, T. (2015). Ecological zonation of benthic foraminifera in the lower Guadiana estuary (south-eastern Portugal). *Mar. Micropaleontol.*, 114, 1–18.

- Cesbron, F., Geslin, E., Jorissen, F. J., Delgard, M. L., Charrieau, L., Deflandre, B., Jézéquel, D., Anschutz, P., and Metzger, E. (2016). Vertical distribution and respiration rates of benthic foraminifera: contribution to aerobic remineralization in intertidal mudflats covered by *Zostera noltei* meadows. *Estuar. Coast. Shelf Sci.*, 179, 23–38.
- Charrieau, L. M., Filipsson, H. L., Nagai, Y., Kawada, S., Ljung, K., Kritzberg, E., and Toyofuku, T. (2018). Decalcification and survival of benthic foraminifera under the combined impacts of varying pH and salinity. *Mar. Environ. Res.*, 138, 36–45.
- Charrieau, L. M., Nagai, Y., Kimoto, K., Dissard, D., Below, B., Fujita, K., and Toyofuku, T. (2022). The coral reef-dwelling *Peneroplis* spp. shows calcification recovery to ocean acidification conditions. *Sci. Rep.*, 12, article no. 6373.
- Cooley, S., Schoeman, D., Bopp, L., Boyd, P., Donner, S., Ghebrehiwet, D. Y., Ito, S. I., Kiessling, W., Martinetto, P., Ojea, E., Racault, M. E., Rost, B., and Skern-Mauritzen, M. (2022). 3: Ocean and coastal ecosystems and their services. In Pörtner, H.-O., Roberts, D. C., Tignor, M., Poloczanska, E. S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., and Rama, B., editors, *Climate Change 2022: Impacts, Adaptation and Vulnerability*, Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, pages 379–550. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Dam, A., Marshall, I. P. G., Risgaard-Petersen, N., Burdorf, L. D. W., and Marzocchi, U. (2021). Effect of salinity on cable bacteria species composition and diversity. *Environ. Microbiol.*, 23, 2605–2616.
- Daviray, M., Geslin, E., Risgaard-Petersen, N., Scholz, V. V., Fouet, M., and Metzger, E. (2024). Potential impacts of cable bacteria activity on hard-shelled benthic foraminifera: implications for their interpretation as bioindicators or paleoproxies. *Biogeosciences*, 21, 911–928.
- Day, J. W., Crump, B. C., Kemp, W. M., and Yañez-Arancibia, A. (2012). *Estuarine Ecology*. John Wiley & Sons, Hoboken, NJ, 2nd edition.
- de Nooijer, L. J., Langer, G., Nehrke, G., and Bijma, J. (2009). Physiological controls on seawater uptake and calcification in the benthic foraminifer *Ammonia tepida*. *Biogeosciences*, 6, 2669–2675.
- Debenay, J.-P., Bicchi, E., Goubert, E., and Armynot du Châtelet, E. (2006). Spatio-temporal distribution of benthic foraminifera in relation to estuarine dynamics (Vie estuary, Vendée, W France). *Estuar. Coast. Shelf Sci.*, 67, 181–197.
- Debenay, J.-P., Guillou, J.-J., Geslin, E., and Lesourd, M. (2000a). Crystallization of calcite in foraminiferal tests. *Micropaleontology*, 46, 87–94.
- Debenay, J.-P., Guillou, J.-J., Redois, F., and Geslin, E. (2000b). Distribution trends of foraminiferal assemblages in paralic environments. In Martin, R. E., editor, *Environmental Micropaleontology: The Application of Microfossils to Environmental Geology, Topics in Geobiology*, pages 39–67. Springer US, Boston, MA.
- Elliott, M. and McLusky, D. S. (2002). The need for definitions in understanding estuaries. *Estuar. Coast. Shelf Sci.*, 55, 815–827.
- Elliott, M. and Quintino, V. (2007). The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Mar. Pollut. Bull.*, 54, 640–645.
- Fouet, M. (2022). *Répartition des communautés de foraminifères dans les estuaires de la façade atlantique*. These de doctorat, Angers.
- Fouet, M. P. A., Singer, D., Coynel, A., Héliot, S., Howa, H., Lalande, J., Mouret, A., Schweizer, M., Tcherkez, G., and Jorissen, F. J. (2022). Foraminiferal distribution in two estuarine intertidal mudflats of the French Atlantic coast: testing the marine influence index. *Water*, 14, article no. 645.
- Francescangeli, F., Milker, Y., Bunzel, D., Thomas, H., Norbistrath, M., Schönfeld, J., and Schmiedl, G. (2021). Recent benthic foraminiferal distribution in the Elbe estuary (North Sea, Germany): a response to environmental stressors. *Estuar. Coast. Shelf Sci.*, 251, article no. 107198.
- Francescangeli, F., Quijada, M., Armynot du Châtelet, E., Frontalini, F., Trentesaux, A., Billon, G., and Bouchet, V. M. P. (2020). Multidisciplinary study to monitor consequences of pollution on intertidal benthic ecosystems (Hauts de France, English Channel, France): comparison with natural areas. *Mar. Environ. Res.*, 160, article no. 105034.
- Guamán-Guevara, F., Austin, H., Hicks, N., Streeter, R., and Austin, W. E. N. (2019). Impacts of ocean acidification on intertidal benthic foraminiferal

- growth and calcification. *PLoS One*, 14, article no. e0220046.
- Haynert, K., Schönfeld, J., Polovodova-Asteman, I., and Thomsen, J. (2012). The benthic foraminiferal community in a naturally CO₂-rich coastal habitat of the southwestern Baltic Sea. *Biogeosciences*, 9, 4421–4440.
- Hermans, M., Lenstra, W. K., Hidalgo-Martinez, S., van Helmond, N. A. G. M., Witbaard, R., Meysman, F. J. R., Gonzalez, S., and Slomp, C. P. (2019). Abundance and biogeochemical impact of cable bacteria in Baltic Sea sediments. *Environ. Sci. Technol.*, 53, 7494–7503.
- Hermans, M., Risgaard-Petersen, N., Meysman, F. J. R., and Slomp, C. P. (2020). Biogeochemical impact of cable bacteria on coastal Black Sea sediment. *Biogeosciences*, 17, 5919–5938.
- Jorissen, F. J., Fouet, M. P. A., Singer, D., and Howa, H. (2022). The marine influence index (MII): a tool to assess estuarine intertidal mudflat environments for the purpose of foraminiferal biomonitoring. *Water*, 14, article no. 676.
- Kuroyanagi, A., Irie, T., Kinoshita, S., Kawahata, H., Suzuki, A., Nishi, H., Sasaki, O., Takashima, R., and Fujita, K. (2021). Decrease in volume and density of foraminiferal shells with progressing ocean acidification. *Sci. Rep.*, 11, article no. 19988.
- Larsen, S., Nielsen, L. P., and Schramm, A. (2014). Cable bacteria associated with long-distance electron transport in New England salt marsh sediment: cable bacteria in New England salt marsh sediment. *Environ. Microbiol. Rep.*, 7, 175–179.
- Le Cadre, V., Debenay, J. P., and Lesourd, M. (2003). Low pH effects on *Ammonia beccarii* test deformation: implications for using test deformations as a pollution indicator. *J. Foraminifer. Res.*, 33, 1–9.
- Malkin, S. Y., Rao, A. M., Seitaj, D., Vasquez-Cardenas, D., Zetsche, E.-M., Hidalgo-Martinez, S., Boschker, H. T., and Meysman, F. J. R. (2014). Natural occurrence of microbial sulphur oxidation by long-range electron transport in the seafloor. *ISME J.*, 8, 1843–1854.
- Malkin, S. Y., Seitaj, D., Burdorf, L. D. W., Nieuwhof, S., Hidalgo-Martinez, S., Tramper, A., Geeraert, N., De Stigter, H., and Meysman, F. J. R. (2017). Electro-genic sulfur oxidation by cable bacteria in bivalve reef sediments. *Front. Mar. Sci.*, 4, article no. 28.
- Meysman, F. J. R., Risgaard-Petersen, N., Malkin, S. Y., and Nielsen, L. P. (2015). The geochemical fingerprint of microbial long-distance electron transport in the seafloor. *Geochim. Cosmochim. Acta*, 152, 122–142.
- Mojtahid, M., Geslin, E., Coynel, A., Gorse, L., Vella, C., Davranche, A., Zozzolo, L., Blanchet, L., Bénétiau, E., and Maillat, G. (2016). Spatial distribution of living (Rose Bengal stained) benthic foraminifera in the Loire estuary (western France). *J. Sea Res.*, 118, 1–16. Recent and past sedimentary, biogeochemical and benthic ecosystem evolution of the Loire Estuary (Western France).
- Moreno, J., Fatela, F., Andrade, C., Cascalho, J., and Drago, T. (2005). Living foraminiferal assemblages from the Minho and Coura estuaries (northern Portugal): a stressful environment. *Thalassas: Int. J. Mar. Sci.*, 21, 17–28. ISSN 0212-5919.
- Nielsen, L. P., Risgaard-Petersen, N., Fossing, H., Christensen, P. B., and Sayama, M. (2010). Electric currents couple spatially separated biogeochemical processes in marine sediment. *Nature*, 463, 1071–1074.
- Pfeffer, C., Larsen, S., Song, J., Dong, M., Besenbacher, F., Meyer, R. L., Kjeldsen, K. U., Schreiber, L., Gorby, Y. A., El-Naggar, M. Y., Leung, K. M., Schramm, A., Risgaard-Petersen, N., and Nielsen, L. P. (2012). Filamentous bacteria transport electrons over centimetre distances. *Nature*, 491, 218–221.
- Redois, F. (1996). *Les foraminifères benthiques actuels bioindicateurs du milieu marin exemples du plateau continental sénégalais et de l'estran du golfe du Morbihan (France)*. Thèse de doctorat, Université d'Angers.
- Revsbech, N. P. (1989). An oxygen microsensor with a guard cathode. *Limnol. Oceanograph.*, 34, 474–478.
- Revsbech, N. P. and Jørgensen, B. B. (1986). Micro-electrodes: their use in microbial ecology. In Marshall, K. C., editor, *Advances in Microbial Ecology*, pages 293–352. Springer US, Boston, MA.
- Richirt, J., Guihéneuf, A., Mouret, A., Schweizer, M., Slomp, C. P., and Jorissen, F. J. (2022). A historical record of benthic foraminifera in seasonally anoxic Lake Grevelingen, the Netherlands. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 599, article no. 111057.
- Risgaard-Petersen, N., Kristiansen, M., Frederiksen, R. B., Dittmer, A. L., Bjerg, J. T., Trojan, D., Schreiber, L., Damgaard, L. R., Schramm, A., and Nielsen, L. P. (2015). Cable bacteria in freshwa-

- ter sediments. *Appl. Environ. Microbiol.*, 81, 6003–6011.
- Risgaard-Petersen, N., Revil, A., Meister, P., and Nielsen, L. P. (2012). Sulfur, iron-, and calcium cycling associated with natural electric currents running through marine sediment. *Geochim. Cosmochim. Acta*, 92, 1–13.
- Schönfeld, J. and Mendes, I. (2022). Benthic foraminifera and pore water carbonate chemistry on a tidal flat and salt marsh at Ria Formosa, Algarve, Portugal. *Estuar. Coast. Shelf Sci.*, 276, article no. 108003.
- Seitaj, D., Schauer, R., Sulu-Gambari, E., Hidalgo-Martinez, S., Malkin, S. Y., Burdorf, L. D. W., Slomp, C. P., and Meysman, F. J. R. (2015). Cable bacteria generate a firewall against euxinia in seasonally hypoxic basins. *Proc. Natl. Acad. Sci. USA*, 112, 13278–13283.
- Sulu-Gambari, E., Seitaj, D., Meysman, F. J. R., Schauer, R., Polerecky, L., and Slomp, C. P. (2016). Cable bacteria control iron–phosphorus dynamics in sediments of a coastal hypoxic basin. *Environ. Sci. Technol.*, 50, 1227–1233.
- Thibault de Chanvalon, A., Geslin, E., Mojtahid, M., Métais, I., Méléder, V., and Metzger, E. (2022). Multiscale analysis of living benthic foraminiferal heterogeneity: ecological advances from an intertidal mudflat (Loire estuary, France). *Contin. Shelf Res.*, 232, article no. 104627.
- Trojan, D., Schreiber, L., Bjerg, J. T., Bøggild, A., Yang, T., Kjeldsen, K. U., and Schramm, A. (2016). A taxonomic framework for cable bacteria and proposal of the candidate genera *Electrothrix* and *Electronema*. *Syst. Appl. Microbiol.*, 39, 297–306.
- Wootton, J. T., Pfister, C. A., and Forester, J. D. (2008). Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proc. Natl. Acad. Sci. USA*, 105, 18848–18853.