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Sediment archives reveal irreversible shifts in plankton communities after World War II and agricultural pollution

Graphical abstract



Highlights

- Protist paleocommunities are mainly composed of resting cysts
- Anthropogenic contamination caused irreversible shifts in protist communities
- Protist division shifts coincided with pollution accumulated during World War II
- Variation in protist genus abundances followed chronic agricultural contamination

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In brief

Using a multidisciplinary paleoecological approach, Siano et al. show irreversible shifts in coastal protist communities coinciding with anthropogenic pollution accumulated during the XXth century. This work highlights the importance of retrospective analyses for the evaluation of marine coastal ecosystem stability and resilience.



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Sediment archives reveal irreversible shifts in plankton communities after World War II and agricultural pollution

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SUMMARY

To evaluate the stability and resilience¹ of coastal ecosystem communities to perturbations that occurred during the Anthropocene,² pre-industrial biodiversity baselines inferred from paleoarchives are needed.^{3,4} The study of ancient DNA (aDNA) from sediments (sedaDNA)⁵ has provided valuable information about past dynamics of microbial species⁶⁻⁸ and communities⁹⁻¹⁸ in relation to ecosystem variations. Shifts in planktonic protist communities might significantly affect marine ecosystems through cascading effects, ¹⁹⁻²¹ and therefore the analysis of this compartment is essential for the assessment of ecosystem variations. Here, sediment cores collected from different sites of the Bay of Brest (northeast Atlantic, France) allowed ca. 1,400 years of retrospective analyses of the effects of human pollution on marine protists. Comparison of sedaDNA extractions and metabarcoding analyses with different barcode regions (V4 and V7 18S rDNA) revealed that protist assemblages in ancient sediments are mainly composed of species known to produce resting stages. Heavymetal pollution traces in sediments were ascribed to the World War II period and coincided with community shifts within dinoflagellates and stramenopiles. After the war and especially from the 1980s to 1990s, protist genera shifts followed chronic contaminations of agricultural origin. Community composition reconstruction over time showed that there was no recovery to a Middle Ages baseline composition. This demonstrates the irreversibility of the observed shifts after the cumulative effect of war and agricultural pollutions. Developing a paleoecological approach, this study highlights how human contaminations irreversibly affect marine microbial compartments, which contributes to the debate on coastal ecosystem preservation and restoration.

RESULTS AND DISCUSSION

Paleocommunity diversity of marine coastal ecosystems

Biological analyses of microeukaryotic (protist) communities were inferred from sediment cores collected at three different sites of the Bay of Brest (Figure S1A) covering about 72 (Elorn Estuary core [EE]; Data S1A), 99 (Daoulas Estuary core [DE]; Data S1B), and 5,000 calendar years (cal. year.) (Brest Harbor core [BH]; Figure S1B; Data S1C; Video S1). The multiple sedimentary ancient DNA (*sed*aDNA) extraction methods combined with a double-metabarcoding analysis that were applied to the BH core provided new information for the future establishment of a

general protocol for marine *seda*DNA studies. After validation on the BH core, this approach was applied to the EE and DE cores. By comparing total (TOT), intracellular (IN), and extracellular (EX) *sed*aDNA extraction methods (Table S1; Figures S2A and S2B) and the α and β protist diversities obtained from two different barcode sizes (V4 [ca. 390 bp; base pairs] and V7 [ca. 200 bp] of the 18S ribosomal DNA [rDNA] gene region) (Table S2), it was found that the TOT *sed*aDNA extraction method allowed the analysis of mostly IN DNA (Figures S2C and S2D). Over the entire BH core, up to 69% of V7 and 58% of V4 amplicon sequence variants (ASVs) that were $\geq 1\%$ abundant in the TOT extracts were also present in the IN fraction (Figure S2E). Our study confirms Armbrecht et al.'s²² hypothesis that



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Figure 1. Paleocommunity diversity

Eukaryotic diversity composition of the BH core of the Bay of Brest inferred by metabarcoding of the V7 (left panel) and V4 (right panel) 18S rDNA of sedaDNA extracted from medieval to contemporary sediments. Eukaryotic lineages are listed and color tones are used to indicate eukaryotic groups.

extraction techniques targeting only EX DNA, such as those used in terrestrial ecological surveys,^{23,24} are not suitable for marine *seda*DNA studies of protists. Given the ability of many protists to form benthic, sedimentary resting stages that protect cellular content—including the nucleus—it is considered that the retrieved IN protist DNA is extracted from resting stages. Indeed, 83% (V7) and 82% (V4) of the common ASVs were assigned to protist genera known to include species that can produce resting stages (Data S2). It is suggested that the amplification of TOT *seda*DNA allows diversity analyses of mostly protist resting stages and that eukaryotic DNA archives in ancient sediments mostly correspond to assemblages of protist resting stages, which are defined here as paleocommunities.

BH sediment samples could be separated into two groups according to ASV richness and β diversities (Figure S2D). This difference was attributed to sedaDNA preservation issues in sandy lower deposits of deeper core layers. Therefore, only the first upper section of the BH core (0-231 cm, ca. 1,400 years from the Middle Ages [Vth to VIth centuries] to the contemporary era [XXIst century]) was considered for biodiversity analyses (Figure S2F). SedaDNA metabarcoding analyses of the BH core showed that the long (V4) and the shorter (V7) fragments provided a similar diversity for eukaryotic paleocommunities (Figure 1). Although only some eukaryotic groups were identified by V7 metabarcoding (unknown Archaeplastida, Telonemia), V4 analysis allowed identifying more groups than V7 (33 versus 27). Therefore, only V4 was considered in the subsequent ecological analyses of the Bay of Brest cores. Degradation of sedaDNA involves DNA fragmentation,²² and therefore the length of the barcode used in metabarcoding influences biodiversity analyses. The amplification of ca. 500-bp fragments from ancient sediment for some protist species was previously reported in ancient sediments (e.g., Coolen et al.¹⁰). In agreement with previous results, here it is demonstrated that ca. 400-bp DNA fragment analyses are also suitable for assessing the diversity of a large fraction of the protist community, confirming that the previous validation of the V4 approach of medieval (until ca. AD 900) to modern sediment from freshwater environments¹³ also works for coastal marine ecosystems.

Paleocommunities of the BH core were mostly composed of three divisions: Alveolata, Opisthokonta, and Archaeplastida, dominated, respectively, by Apicomplexa (average: 33%), Metazoa (12%), and Streptophyta (8%). Dinoflagellata (Alveolata) (6%) were the fourth abundant group. Unassigned eukaryotic ASVs (unknown) accounted for on average 28% of the paleocommunity biodiversity (Figure 1, right panel). The relative importance of the most important groups varied throughout the core. Metazoa largely dominated in superficial sediments (3-11 cm; average: 45%; max: 70%) with Annelida ASVs as the most representative phylum (average: 38%). The protist group of Apicomplexa usually dominated throughout the rest of the BH core (13-231 cm; average: 30%; max: 59%; Figure 1) and the EE and DE cores (Figure S3). Within this group the invertebrate parasitic class of gregarines was prevalent (average: 25%; max: 55% of total eukaryotes), dominated by three genera of invertebrate coelomic parasites²⁵ (average, max: Lecudina, 15%, 50%; Lankesteria, 2%, 8%; Selenidium, 1%, 8%). Dinoflagellates were occasionally abundant both in superficial (22%, 23%) and in deeper sediments (14%, 20%).

Paleocommunities differ from both superficial sediment and planktonic protist communities. In plankton communities, the supergroup of Alveolata (specifically marine alveolates [MALVs] and dinoflagellates) dominate together with Rhizaria and stramenopiles (particularly Bacillariophyta), respectively, the offshore²⁶ and the coastal protist communities.²⁷ Superficial sediment communities are dominated by Rhizaria (in particular Cercozoa), Alveolata (Ciliophora), and stramenopiles (Bacillariophyta), and share <10% of the species richness with the planktonic community.²⁸ The dominance or high proportion of gregarines, a resting-stage-forming group of metazoan parasites,²⁹ can be

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Figure 2. Shifts in dinoflagellate community composition

Dinoflagellate diversity analyses inferred from the V4 metabarcoding data of the TOT sedaDNA of the BH, DE, and EE cores of the Bay of Brest. Core dinoflagellate orders are identified with color tones (red, Gonyaulacales; yellow, Peridiniales; blue, Gymnodiniales; green, Suessiales; violet, Dinophysales). The dinoflagellate sister clades of marine alveolate (MALV) groups I, II, and III are also represented. Unidentified dinoflagellates are classified as unknown. The multivariate regression tree analysis suggested dates of chronological partitioning of the data, representing shifts in community composition, and identified the dinoflagellate group mostly explaining these shifts. Only the first and major complexity level is represented. The genus *Gonyaulax* (Gonyaulacales) is responsible for 100% of the deviance in the break point identified in 1941 for BH (complexity: 35.6), for 43% of the deviance in the break point identified in 1998 for EE (complexity: 33.7). See also Figures S2 and S3.

considered a specific protist biodiversity pattern of marine sediment archives. Indeed, Apicomplexa were also found abundant in marine sedimentary archives of the Arabian Sea and associated with depleted oxygen conditions.¹⁵ Gregarines in particular were dominant in soil protist communities of Neotropical forests, findings that suggest those groups shape animal diversity in forest ecosystems.³⁰ Similarly, these parasites could contribute to shaping marine animal communities in a marine ecosystem. Their dominance in centuries-old protist paleocommunities could suggest long-term parasitic pressure over animal communities. Hence, gregarines may have a trans-ecosystem functional role, and protist paleocommunities, in both soils and marine sediments, might reveal long-term host-parasite ecological patterns.

Paleocommunity shifts in relation to coastal pollution

The multivariate regression tree (MRT) analysis suggested that shifts in the protist communities occurred during the 1930s to 1940s and 1980s to 1990s (Figure S3). The protist groups largely responsible for these shifts were dinoflagellates (Figure 2) and stramenopiles (Figure 3).

Dinoflagellate communities drastically changed during the 1940s (break point 1941, BH core), shifting from the dominance of suessioid genera (Pelagodinium and Biecheleria/Protodinium; Figure 2) during the entire Middle Ages and the XIXth century to the beginning of the XXth century to a dominance of gonyaulacoid genera (Gonyaulax and Alexandrium; Figure 2) during the end of the XXth and beginning of the XXIst centuries. Suessioid genera almost disappeared after the 1980s (1987, DE); they are indeed relatively rare in current coastal plankton,²⁷ and peridinioid genera became more important (Figure 2). Changes in the relative importance of the genus Gonyaulax largely explain dinoflagellate variations. The genus Gonyaulax progressively decreased in importance after the 1980s (1987, DE) and the 1990s (1998, EE), whereas the genera Heterocapsa (peridinioid) and the potentially harmful genus Alexandrium increased in importance (Figure 2). This result confirms previous observations of increased abundance of the toxic species Alexandrium minutum revealed by real-time PCR analyses of sedaDNA from the same DE and EE cores in parallel with the recrudescence of harmful algal blooms caused by this species over time in the Bay of Brest.

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Figure 3. Shifts in stramenopile community composition

Stramenopile diversity analyses inferred from the V4 metabarcoding data of the TOT sedaDNA of the BH, DE, and EE cores from the Bay of Brest. Stramenopile groups are identified with color tones (red, phylum Bacillariophyta [diatoms]; green, phylum Dictyochophyta; yellow, phylum Labyrinthulea; blue, marine stramenopiles [MAST]; violet, Oomycota; other colors, clades with *incertae sedis* classifications). Unidentified stramenopiles are classified as unknown. The multivariate regression tree analysis suggested dates of chronological partitioning of the data, representing shifts in community composition, and the identified stramenopile group mostly explains these shifts. Only the first and major complexity level is represented. The diatom genus *Chaetoceros* is responsible for 93% of the deviance in the break point identified in 1952 for BH (complexity: 19.5) and 100% of the deviance in the break point identified in 1998 for EE (complexity: 33.7). The group MAST-12A is responsible for 100% of the deviance in the break point identified in 1945 for EE (complexity: 33.7).

Stramenopiles also drastically shifted during the 1940s and 1950s (Figure 3). The heterotrophic group MAST (marine stramenopiles³¹) and in particular the MAST-12 clade dominated stramenopile paleocommunities during the Middle Ages and the XIXth century (Figure 3). The relative abundance of Bacillariophyta (diatoms) started to increase during the XXth century (1942, DE) and dominated after the 1950s (1952, BH) with MAST-12 progressively decreasing in abundance (Figure 3). Within the diatom community, *Chaetoceros* was progressively replaced by the genus *Thalassiosira* as well as by other unidentified diatoms after the 1990s (1998, EE) (Figure 3).

As noted, genera found in pre-war sediments became very rare after the World War II (WWII) period and conversely current dominant genera were rare in the past. This indicates that the community shifts observed in this study were irreversible at the timescale of our paleogenetic analyses.

To infer potential causes of paleocommunity shifts in the Bay of Brest, contaminants were analyzed in sediment samples of the BH core. Three groups of heavy metals were discriminated in sediment samples (non-parametric correlation, Spearman

aluminum (Al)-normalized lithium (Li), nickel (Ni), mercury (Hg), and lead (²⁰⁶Pb/²⁰⁷Pb) were examined in the sediment samples (Figures 4 and S4D) as representative of each of the three groups. Li-normalized concentrations and dynamics were constant across the studied interval (12.9 \pm 0.8 mg×kg⁻¹; mean \pm SD). Ni had constant normalized concentrations throughout the core $(4.8 \pm 0.2 \text{ mg} \times \text{kg}^{-1})$ with the exception of two peaks detected in the years 1947 \pm 11 (WWII period) and 1913 \pm 16 (WWI period) (a concentration increase of up to 50%, 9.8 mg×kg⁻¹) (Figure 4). At 1936 \pm 12 (WWII period), a small ²⁰⁶Pb/²⁰⁷Pb peak was also detected. Hg increased its normalized concentrations from 1958 ± 9 to more recent times (surface layers) with a peak at 1981 \pm 5 (Figure 4). To evaluate whether pollutant sources were of an anthropogenic origin, the relative enrichment factor (EF) was calculated for every trace metal. The two concentration peaks observed at 1947 ± 11 for Ni (Figure 4) and Cr (Figure S4B) correspond to a significant anthropogenic impact (EF = 2.0 for Ni). The EF and concentrations of Hg (Figure 4) and other trace metals of group 3 (Figure S4C)

test, p < 0.05; Figures 4 and S4A-S4C) and concentrations of

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Figure 4. Pollutant temporal variations

Pb isotope ratio and selected heavy-metal and PCB concentrations in the BH core of the Bay of Brest. The Al-normalized dynamics of Li, Ni, and Hg are representative of three groups of analyzed heavy metals. The cumulative concentrations of the seven measured indicator PCB congeners (CB28, 52, 101, 118, 138, 153, 180) are indicated as Σ7PCBs. See also Figure S4.

progressively increased over time, with maximum EF values between 1.9 and 9.5. These values demonstrated a moderate to severe anthropogenic contamination after WWII in this order: Zn < Pb < Ag < Cu < Hg. This post-WWII pollution dynamics was confirmed by Pb isotope ratios (Pb/AI, ²⁰⁶Pb/²⁰⁷Pb, ²⁰⁶Pb/²⁰⁸Pb; Figures 4, S4D, and S4E) and polychlorinated biphenyl (PCB) contamination profiles (Figure 4). The concentration profile of the sum of the seven PCB indicator congeners confirmed the influence of higher anthropogenic input from the 1950s onward. Indeed, the concentration profile of the sum of the seven indicator congeners showed a progressive increase in PCB contamination from 1958 ± 7 until the peak at 1981 ± 5 following the pattern of the third group of heavy metals (Figure 4).

During WWII the town of Brest was under Nazi occupation. Historical documents provide qualitative information about wartime activities linked to pollution, such as the construction of a submarine base, industrial army activity, navy traffic, and fuel deposit fires. Historical data are available about the quantity of bombs dropped on the town of Brest and its bay. The 30,000 tons of bombs and the 100,000 shells deployed over Brest during WWII and even more intensely during Allied liberation (Figure S1C) must have polluted the water either directly or indirectly through waste and detritus collected by river catchments flowing into the coastal waters of the bay. The metal composition of such bombs and explosives is imprecise or very difficult to retrieve. Yet, it is interesting that trace metal anomalies, including Pb and Cr, were found in sediment samples to be coincident with the Japanese aviation bombing of Pearl Harbor, the U.S. navy outpost during WWII, on December 7, 1941.32

Regarding the post-WWII period it is difficult to designate a particular source of metal contamination and the potential inputs could come from atmospheric depositions (Hg and Pb), agricultural phytosanitary products (Cu and Zn), and urban waste (polymetallic inputs). PCB contamination profiles sustain the hypothesis of contaminations of agricultural origin from the 1950s onward. These profiles in sediment are similar to those of other French human-impacted sites^{33,34} or other agriculturally exploited sites.^{35,36} A palynological study (pollen grains and dinoflagellate cysts) carried out on a twin core of the DE allowed

direct comparison between the evolution of landscape, surface water, and human practices in Bay of Brest watersheds and corroborate the hypothesis of post-war agricultural contamination.³⁷ Pollen and dinoflagellate cyst concentration analyses indicate changes in agricultural practices in the Bay of Brest. An increase in *Alnus* pollen grain concentrations and shifts between cysts of *Lingulodinium polyedra* and *Gonyaulax digitale* were synchronous with protist shifts shown in this study. These variations have been associated with the evolution of agricultural practices and the increase in river flow loading due to the implementation of a new agricultural policy after 1945.³⁷

Plankton shifts have been evaluated on relatively short timescales because the majority of plankton long-term series have been initiated from the 1950s onward, with the exception of the Continuous Plankton Recorder Survey, which started in 1937.³⁸ Changes in plankton communities during the 1980s to 1990s were already observed in the Bay of Brest using microscopy counts. These shifts were associated with unbalanced nitrogen/ phosphorus (N/P) ratios over time and in particular with the high concentrations of nitrogen in coastal water due to run-offs of agricultural fields through water catchments and rivers.^{39,40} To compensate for the gap in pre-industrial plankton data, researchers have used modeling approaches mostly to evaluate the effect of climate changes on plankton.41,42 Traditional sedimentary paleoecological approaches based on microfossil analyses (spores, diatom frustules, dinoflagellate cysts, foraminifers and coccoliths, and acritarchs) have allowed researchers to evaluate shifts on a paleoclimatological timescale but also community changes after industrial pollution.^{37,43} SedaDNA analyses of plankton have shown its potential as a tool for studying the effect of human perturbation on biological communities.^{18,43-46} Paleogenetic analyses revealed long-term shifts in plankton sometimes proving anthropogenic influence^{9,10,12,13,17} and the irreversibility of those changes. 47,48 SedaDNA analyses have highlighted the increase and recrudescence of harmful algal bloom species in lakes⁴⁹ and coastal waters^{7,8} and reflected an increase in eutrophication due to human pressures.^{9,10,13,17,48,50} Our paleogenetic study showed that the cumulative effect of the trace metal and PCB pollution of the WWII and the post-war periods caused

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drastic changes in paleocommunity compositions, especially for dinoflagellates and stramenopiles, and the variations in dominant genera within these groups after the 1980s and 1990s. The effects of punctual or long-term contaminations from heavy metals (including Pb, Ni, and Cr) and PCBs are known to affect coastal plankton community composition^{51,52} and to have a negative effect on phytoplankton species development,⁵³ including dinoflagellates.^{54,55} The occasional and chronic human impacts that occurred during the WWII and post-war period resulted in unstable and non-resilient variations of protistan communities in the Bay of Brest over the last 70 years. Hence, this study demonstrates the potential irreversible consequence of multiple and cumulated pollutions for the microbial compartment of the coastal ecosystem.

What defines healthy coastal ecosystems is under debate. The main issue is whether natural systems are in balance and whether they tend to return to that balance when perturbed or whether ecosystems are dynamic systems that are transient and unstable.¹ The bottleneck of such analysis is the contemporary acquisition of baselines of pristine ecosystem conditions and post-perturbation analyses. The comparative analysis of past versus modern ecosystem conditions may help to establish or optimize ecosystem strategies.⁴ This study highlights the importance of retrospective analyses for the evaluation of coastal ecosystem stability and resilience and the relevance of paleogenetic data for these analyses. The impact of short- and long-term chronical pollution is demonstrated here on microeukaryotic planktonic communities, a key compartment in marine ecosystems. Shifts in this compartment might cause cascading effects on other biological components of the ecosystem affecting the whole marine food web. These findings can further sustain the use of the paleoecological approach for the development of restoration efforts and policy actions aiming at a sustainable management of coastal areas.

STAR * METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
 - Lead contact
 - Materials availability
 - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
 - Study area
 - Core sediment sampling
- METHOD DETAILS
 - Sediment dating and granulometry
 - sedaDNA extraction and amplification
 - Metabarcoding analyses
 - Pollutant analyses
 - Historical archive documentation
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j. cub.2021.03.079.

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AUTHOR CONTRIBUTIONS

R.S. designed and performed the research, conceived and carried out the sampling, analyzed the results and data, and wrote the paper. M. Lassudrie performed the DNA extraction and genetic analyses, analyzed the results, and contributed to writing. P.C., C.N., and L.Q. contributed to the metabarcoding biostatistical analyses. P.D. curated the data depository. N.B. and V.L. performed, respectively, the heavy-metal and PCB analyses, analyzed the results, and contributed to writing. S.S. carried out the sediment dating and contributed to writing. A.E. helped in core sampling realization, carried out the granulometry analyses, and contributed to writing. K.N.M., C.L., and A.P. contributed to the paleoecological data analyses and interpretation. M. Lassudrie and J.Q. technically supported the sampling and genetic analyses.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR***METHODS**

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw Illumina-Miseq data used for metabarcoding analyses	https://www.ncbi.nlm.nih.gov	PRJNA667629 (BH data)
Raw Illumina-Miseq data used for metabarcoding analyses	https://www.ncbi.nlm.nih.gov	PRJNA680674 (DE and EE data)
Chronology of the EE and DE cores	7	https://doi.org/10.1093/femsec/fiw101; Figure 2
Oligonucleotides		
Primer Forward with GeT-PlaGe adaptor CTT TCC CTA CAC GAC GCT CTT CCG ATC TCC AGC ASC YGC GGT AAT TCC	This paper	TAReuk454FWD1- GeT-PlaGe
Primer Reverse with GeT-PlaGe adaptor GGA GTT CAG ACG TGT GCT CTT CCG ATC TAC TTT CGT TCT TGA TYR A	This paper	TAReukREV3- GeT-PlaGe
Primer Forward with GeT-PlaGe adaptor CTTTCCCTACACGACGCTCTTCCGATCT GGCTTAATTTGACTCAACRCG	This paper	960F-GeT-PlaGe
Primer Reverse with GeT-PlaGe adaptor GGAGTTCAGACGTGTGCTCTTCCG ATCTACTGGGCATCACAGACCTGTTAT	This paper	NSR1438-GeT-PlaGe
Software and algorithms		
Calib Rev 7.0.4	56	https://doi.org/10.2458/azu_js_rc.55.16947
R Project for Statistical Computing, vers. v3.4.4	57	https://www.r-project.org/
Cutadapt v1.18	58	https://doi.org/10.14806/ej.17.1.200
BBmap v38.22	This paper	https://jgi.doe.gov/data-and-tools/bbtools/
DADA2 R package	59	https://doi.org/10.1038/nmeth.3869
PR2 v4.11	60	https://doi.org/10.1093/nar/gks1160
vegan R package	61	http://cran.r-project.org/, http://vegan.r-forge. r-project.org/
phyloseq R package	62	https://doi.org/10.1371/journal.pone.0061217
Dplyr and Tidyr R packages	57	https://www.r-project.org/
ggplot2 R package	63	Springer International Publishing)
Mvpart R package	64	http://cran.r-project.org/web/packages/mvpart/ index.html
Other		
Methods video	This paper	Video S1

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Raffaele Siano (raffaele.siano@ifremer.fr).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data needed to evaluate the conclusions of the paper are presented in the paper and/or in the Supplemental information. Raw Illumina-Miseq data used for metabarcoding analyses have been submitted to NCBI (https://www.ncbi.nlm.nih.gov) with the data reference number PRJNA667629 (BH data) and PRJNA680674 (DE and EE data).

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Current Biology Report

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study area

The Bay of Brest (Brittany, France, northeast Atlantic) is a semi-enclosed coastal ecosystem connected to the Iroise Sea (Atlantic Ocean) by a narrow (2 km) and deep (50 m) strait (Figure S1A). The bay is a shallow macrotidal coastal ecosystem with 50% of its surface deeper than 5 m. It is characterized by coarse sediments in deep waters and fine and muddy sediments in the upstream part of the estuaries. The majority (80%) of freshwater inputs come from two main rivers: the Aulne River (1842 km²) in the southeastern part and the Elorn River (402 km²) in the northeastern part of the Bay. The Brest area experienced a progressive development from the Antiquity through the Middle Ages during the Duchy of Brittany, but it became a real naval town during from the XVIIth to XVIIIth centuries with the construction of the naval harbor. During this time, the population and the naval activity increased progressively. Then, after a lull of almost a century, Brest experienced a second industrial development from the end of the XVIIIth century until World War I. In 1940, the German army conquered Brest and built the famous submarine base in only three months. The base, the coastal area, and the whole town were bombed continuously by the Allies until 1945, with more than 30000 tons of bombs deployed (Figure S1C). The town of Brest was completely destroyed, with 965 people killed and 740 injured. During this period, the coastal area was the receptacle of heavy pollution due to the industrial army activity, navy traffic, and fires of French fuel deposits practiced by the German army on the land. The town of Brest was liberated on September 19, 1944, after 45 days of heavy fighting. After the liberation, the town and the harbor started their reconstruction. In 1947, the explosion of the Ocean Liberty vessel provoked grave damage to the entire town and the coastal area, with more than 3000 tons of the heavily explosive fertilizer ammonium nitrate released in the harbor's waters. Since 1950, the Brest area has undergone an intensive agricultural industrial development: the urban area increased and the harbor extended over the sea. During this period and until the 1990s, the area had experienced several changes in the anthropogenic loading of nutrients; with an increase in nitrogen supply due to the development of anthropogenic activities, and near the end of that period, a decrease in phosphorous supply resulting from the ban of washing powders containing orthophosphates.⁶⁵ Consequently, a significant imbalance in the nitrogen/phosphorus ratio⁶⁶ altered the composition of planktonic and benthic communities.^{39,40,67} After the 1990s, the agricultural policy of the region has tried to control eutrophication of the area, and since then the nutrient loading has progressively decreased in the coastal area of the Bay of Brest.

Core sediment sampling

Biological analyses were inferred from three sediment cores collected in the Bay of Brest. Two interface cores were collected by a gravity corer at the main estuaries' outlets of the Bay of Brest: the Elorn Estuary (EE: 48°23'46.79"N, 4°23'2.01"W; December 11, 2012; 12 m depth; core length 31 cm) and the Daoulas Estuary (DE: 48°20'46.6"N, 4°17' 41.20"W; May 21, 2014; 3 m depth; core length 58 cm) (Figure S1A). A third core was collected near the Brest Harbor in a non-dredged zone (BH: 48°22'52.74"N, 04°26'54.60"W; April 25, 2017; 7.10 m depth; core length 344 cm) on-board the N/O Thalia using a Kullenberg core sampler (Figure S1A; Video S1). Dating was obtained from a twin core collected at the EE (see Klouch et al.⁷ for details of methods) and from the same cores used for biological analyses at DE and BH. Immediately after sampling, sediment cores were delicately extruded and sliced into 1 cm thick layers (Video S1). Due to its length, the BH core was previously separated into four sections of equal length. For EE and DE interface cores, every 1 cm layer was collected and analyzed. For the long BH core, only one in two slices was used for subsampling and further analyses to avoid contamination across sample layers. To further avoid contamination by smearing between layers during the core extraction, only the inner part of each slice was subsampled, using sterile 6 cm diameter Petri dishes and sterilized wooden tongue depressors (Video S1). Sterile, new equipment was used to sample each layer. Samples collected in a Petri dish for genetic analyses (about 10 g of wet sediment) were preserved in plastic 50 mL deoxygenated cryotubes, immediately frozen in liquid nitrogen, and then stored in a -80° C freezer until further analyses. Samples for granulometry, organic carbon, heavy metals, and PCB compounds were collected from the same layers of DNA samples, whenever possible, or from the discarded layer not used for genetic analyses. For organic carbon analysis, samples were frozen at -20°C and then lyophilized before analysis. Other sediment aliquots were stored at 4°C and in the dark until further analysis.

METHOD DETAILS

Sediment dating and granulometry

Dating of recent sediments (< 150 years) was established for the Elorn Estuary (EE), Daoulas Estuary (DE) and Brest Harbour (BH, KS06) cores using depth profiles of excess ²¹⁰Pb (²¹⁰Pb_{xs}, lead), a naturally-occurring radionuclide, and ¹³⁷Cs (cesium), an artificial radionuclide. The chronology of DE and EE were previously described by Klouch et al.⁷ The EE and DE cores covered, respectively, about 72 cal. year. (calendar year) (from 1939 ± 2 to 2011 ± 1 AD) and 99 cal. year. (from 1915 ± 7 to 2014 ± 1 AD) (Data S1A and S1B). The BH core is here described for the first time (Figure S1B; Data S1C) and covers the longest time period from 2928 ± 94 BC (4878 ± 94 BP) to 2015 ± 1 AD, i.e., about 5000 cal. year.

For the BH core activities (mBq g⁻¹) of ²¹⁰Pb, ²²⁶Ra (radium), and ¹³⁷Cs were measured in ~10 g of dry sediment using Broad Energy Germanium γ detector (BEGe, Mirion Technologies, France). The presence of shell fragments and maerl made gamma measurements difficult. First, the coarse carbonate fraction was sorted and removed. The remaining fine carbonate fraction diluted the radioelement contents, which implies long counting times ranging from 24 to 126 hours per sample. The detector was calibrated using certified reference material from the International Atomic Energy Agency (RGU-1; SOIL-6). Excess ²¹⁰Pb (²¹⁰Pb_{xs}) was calculated

Current Biology Report



as the difference between the measured ²¹⁰Pb and ²²⁶Ra activities. Activity errors were based on 1 SD counting statistics and propagated for ²¹⁰Pb_{xs}. The two first layers of the core were measured twice a few days and several months after sampling to determine excess ²³⁴Th (thorium), a short-lived radionuclide (T_{1/2} = 24.1 days), which is a tracer of a good recovery of the uppermost sediment and of bioturbation. Activities of ²¹⁰Pb_{xs} present an exponential decrease from the top of the core to negligible values below 20 cm. This rapid decrease and the absence of a mixed layer in the upper section of the ²¹⁰Pb_{xs} profile testify to the absence of significant mixing of the sediment. The long-lived and naturally-occurring ²³²Th is usually associated with the detrital fraction; therefore, the rather constant ²³²Th activity in the BH core indicates the absence of changes in lithological sources or proportions. This implies that the changes in ²¹⁰Pb_{xs} activities with depth are mainly related to time (radioactive decay of ²¹⁰Pb according to its half-life of 22.3 years) and are suitable to estimate sedimentation intensity.

The constant flux and constant sedimentation (CF–CS) model were used to calculate a mean sedimentation accumulation rate of 0.23 ± 0.040 cm year⁻¹ from the ²¹⁰Pb_{xs} profile. The DE and EE cores have higher mean sediment accumulation rates due to fluvial sediment inputs (EE: 0.44 cm year⁻¹; DE: 0.41 cm year⁻¹) than the BH core. For BH the ages of the sediment layers were calculated using the sedimentation rate and assuming the age of the top sediment to be 2015. The ¹³⁷Cs activities are low but present an onset at about 16 cm, which corresponds to the first introduction of this artificial radionuclide in the environment in the early 1950s and validates the chronological framework based on ²¹⁰Pb_{xs} (Figure S1B).

Dating of ancient sediments (> 150 years) for BH was performed by analyzing ¹⁴C in eight gastropod shells (Bittium spp., Crisilla spp., Hydrobia spp., Jujubinus spp., and Rissoa spp.; total weight of each sample < 10 g) collected from the sieved sediment. The remaining material of five of these samples was not enough to perform genetic extractions. ¹⁴C analyses were conducted at the Poznań Radiocarbon Laboratory (Poznań, Poland). Absolute dating was corrected for the mean ¹⁴C age difference between the atmosphere and oceanic surface waters by applying a reservoir correction (R) of 325 years with an applied regional deviation (DR) for the bay of Brest of 46 years. The results of absolute dating were then calibrated using Calib Rev 7.0.4 software from the "Intcal13" calibration curve,⁵⁶ with a confidence level of 95% for the SD (sigma 2). There was no dating inversion for the eight analyzed samples; however, the ¹⁴C results showed two important time gaps in dating (Data S1C). The former concerns the gap between 32 and 82 cm below the seabed surface separating the recent sedimentation developed from the middle of the XIXth century (1879 year. ± 21 AD), estimated by ²¹⁰Pb_{xs}, and old deposits dated from the central middle age (530 ± 108 AD to 874 ± 111 AD). Additional sediment analyses on other cores (data not shown) showed that this gap is less important and may have occurred between 1000 AD and 1350 AD. This time offset is recorded for all the conserved sediment deposits in the bay of Brest. This period is well known to be a warm climatic sequence called the Medieval Warm Period (MWP) characterized by recurring winter successive storms, 56 at the origin of the construction of aeolian dune systems on the North Atlantic Ocean coasts. The second time gap separates the Middle Ages from the Egyptian period, more precisely corresponding to the Third Intermediate Period until the Early Dynastic Period. It represents a seismic unconformity surface ranging from 2025 ± 129 BP to 3184 ± 164 BP⁵⁶ and corresponds to be a typical geological transition characterizing a precise step of the marine transgression and called the maximum flooding surface (MFS). The coarse deposits between 213 and 233 cm in the core associated with this time gap result of a high-energy marine environment that was able to erode underlying sand Egyptian age deposits.

For sediment granulometry, particle size analyses were carried out with a laser diffraction system for the sediment fraction below 2 mm. The percentages of the main sedimentary classes (gravel, sand, silt, and clay) were thus determined for each level sampled with the Gradistat package developed by Gregoire et al. (see Gregoire et al. ⁶⁸ for methods). All cores mostly comprised a mix of clay, silt, and fine sand particles; the BH core was $4 \pm 4\%$ clay, $43 \pm 9\%$ silt, and $36 \pm 8\%$ fine sand particles (mean \pm standard deviation [SD]). However, in the lower part of the BH core, below 204 cm, the sand and detritic gravel sediment fraction increased to represent more than 80% of the total sediment sampled.

sedaDNA extraction and amplification

Genomic DNA extraction was carried out from 3–10 g of wet sediment from each of the 31 and 58 layers, respectively, of the EE and DE cores, and from 46 layers of the BH core (Table S1). In marine sediments, given the importance of resting stages – which protect DNA inside the cells – it was hypothesized that intracellular DNA constitutes the highest proportion of the total *sed*aDNA extracted with classical protocols used for soil matrices, such as in the DNeasy PowerMax Soil Kit (QIAGEN). This hypothesis was tested on the BH samples. For each of the 46 sediment layers of BH, three different extraction protocols were applied to extract respectively: (1) total DNA (TOT) (DNeasy PowerMax Soil Kit as per the manufacturer instructions); (2) intracellular DNA (IN) from resting stages, ⁶⁹ by eliminating extracellular DNA through washing and heating steps before DNA extraction using the DNeasy PowerMax Soil Kit); and (3) extracellular DNA (EX) (following methods used in terrestrial ecological biodiversity surveys, ²⁴ which comprises extracting extracellular DNA in saturated phosphate buffer prior to using the DNeasy Power Max soil kit without the cell lysis step).

All sedaDNA extractions and manipulations were performed to avoid cross-contamination between samples or with modern DNA, by following specific instructions for working with aDNA and specifically for marine sedaDNA.^{5,22,70} Extractions were carried out in an isolated, specific clean laboratory adapted to aDNA extraction, under a laminar flow cabinet (Video S1). One sediment layer was extracted at a time, all the surfaces and material used were carefully decontaminated between each sample treatment using chlorine or DNA-away (Thermo Fisher), and the laminar flow cabinet was also decontaminated with ultraviolet light. The analyst wore dedicated clothing and disposable personal protective equipment including a lab coat, gloves, and a facemask that were changed between each sample (Video S1). Blanks (no template controls) were extracted to check for the absence of contamination from the working

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Current Biology Report

area and from kit reagents. DNA extracts were immediately stored in a dedicated -80°C freezer. DNA was quantified with the QuantiT PicoGreen dsDNA assay (Invitrogen).

IN sedaDNA extractions yielded a DNA concentration close to that of total TOT sedaDNA extractions all along the BH core (Figures S2A and S2B), whereas EX sedaDNA extractions yielded a constant, low DNA concentration all along the core (Figure S2A; Table S1). There were higher concentrations at superficial core layers in TOT and IN fractions (5 cm depth; max 4971.61 ng DNA g^{-1}), then a rapid decrease in concentrations down to 19 cm depth (max 761.64 ng DNA g^{-1} in TOT). There were constant TOT and IN sedaDNA concentrations from 19 to 222 cm depth (respective averages \pm standard deviation: 697 \pm 135 and 566 \pm 113 ng DNA g^{-1}). From 231 cm to the end of the core, TOT and IN sedaDNA concentrations decreased considerably (278 \pm 80 and 239 \pm 94 ng DNA $g^{,1}$ Figure S2A). This section of the core corresponded to coarser (sandy) sediment granulometries compared with the rest of the core. Such a sediment type is less favorable to DNA preservation, which probably explains the DNA concentration protocol was used for EE and DE extractions. The comparison of different DNA extraction methods showed that the DNA concentrations in sediments along the BH core were similar when comparing total sedaDNA (TOT) and intracellular sedaDNA (IN) extracts, whereas the concentration was much lower in the extracellular sedaDNA (EX) extracts (Table S1).

To analyze possible sedaDNA degradation by fragmentation and to check the possibility and reliability of the amplification of > 300 bp DNA fragments from ancient marine sediments, metabarcoding analyses of the V4 (ca. 390 bp) and V7 (ca. 260 bp) hypervariable regions of the 18S rRNA gene were compared for BH samples. The V4 region is commonly used in protist biodiversity survey,^{27,50} whereas the V7 barcode has been suggested as a useful paleogenetic barcode in paleolimnological studies,¹³ although it had not been tested in marine paleogenetic approaches. The V4 and V7 regions were amplified in samples and extraction using respectively the eukaryotic primers TAReuk454FWD1 and TAReukREV3²⁷ and 960F et NSR1438¹³ assembled with the GeT-PlaGe adaptors from GeT sequencing platform (Toulouse, France, http://get.genotoul.fr) for Illumina Mi-Seg sequencing. For both amplifications, the reaction mixture was prepared in a final volume of 50 µL, containing 0.02 U µL⁻¹ Phusion High Fidelity DNA polymerase and 1X Phusion High-Fidelity Buffer (NEB), 0.2 mM dNTP mix, 0.4 µM of each primer, 3% dimethyl sulfoxide, 2 µL DNA template, and DNA-free water. Extraction blanks were also amplified, and positive controls (mock protist community composed of five different species of microalgae) and negative controls (without DNA templates) were used to check the efficiency and specificity of the PCR. The following cycling program was used for V4: an initial denaturation step at 98°C for 30 s, 14 cycles of 98°C for 10 s, 53°C for 30 s, and 72°C for 30 s; 21 cycles of 98°C for 10 s, 48°C for 30 s, and 72°C for 30 s; and a final elongation step at 72°C for 10 min. The cycling program for V7 was: an initial denaturation step at 98°C for 30 s; 35 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 30 s; and a final elongation step at 72°C for 10 min. PCR products were checked on a 1.5% agarose gel (1 × Trisborate-ethylenediaminetetraacetic acid and Gel Red). Three replicates reactions per sample were run and PCR products were pooled and sent to GeT sequencing platform, where they were purified and checked for quality and quantity using a fragment analyzer. PCR products yielding an insufficient DNA concentration of the expected fragment size were excluded (extraction blanks, 3 EE samples, 27 DE samples, 7 EX DNA, and 1 IN DNA for the V4 region in BH samples). Four libraries were built, keeping separate the samples from different cores and the different regions (V4 and V7), for sequencing from both sides using an Illumina MiSeq (2 × 250bp for V4 and 2 × 150bp for V7). As for the BH core, for the V4 region the range number of reads obtained after sequencing were: 13 812 to 143 391 (TOT), 32 487-118 101 (IN), and 7 566-129 794 (EX). For the V7 region the range number were: 2 585 to 72 950 (TOT), 1795-73 061 (IN), and 997-112 916 (EX). For the EE and the DE core the V4 read number range were respectively: 32 734-425 286 and 37 471-236 421.

Metabarcoding analyses

Sequenced data of the V4 (BH, DE and EE) and V7 (BH) 18S rDNA were submitted to quality checking by FastQC coupled with MultiQC. Primers and adapters have been removed in forward and reverse file separately with Cutadapt v1.1858 using the "-discard-untrimmed" argument, which discards reads without target primers. BBmap v38.22 was used to reorder reads and eliminate singletons from each pair with the repair function. Sequence filtering and trimming as well as Amplicon Sequence Variant (ASV) inference were carried out using the DADA2 strategy with the DADA2 R package and using the default parameters.⁵⁹ Taxonomic assignment of ASV was performed on the basis of the naive Bayesian RDP methodology with the database PR² v4.11.⁶⁰ Finally, metadata files (sample ID, sediment layer dept, type of DNA extraction, and taxonomy) was separately created for each sediment core (BH, DE, and EE). There was a second data cleaning to remove ASVs identified as contamination (e.g., ASVs assigned to Homo sapiens or Capra hircus). For diversity and ecological studies, ASVs with relative abundances < 0.0% for the V4 dataset and < 0.006% for the V7 dataset were removed from the databases using the R package phyloseq. This step allowed us to eliminate rare ASVs and further sequence errors and to analyze > 90% of total ASV richness for each database (Table S2). After this step, samples 1-4 (6 cm depth) and 1-6 (8 cm) of the BH core were eliminated from the V4 and V7 database because the observed richness value was too low. To compare samples, V4 and V7 data of all cores were normalized by a rarefaction method, which comprises a random subsampling of the data by a hypergeometric law. A normalization threshold was established on the basis of rarefaction curves (Figure S2F) modeled using the function ggrare deriving from the function rarecurve of the vegan R package with the argument "step=100."61 The phyloseq R package rarefy_even_depth was used for data normalization.⁶² For BH, only samples from the surface to 231 cm, used for diversity and ecological analyses, were normalized. For DE and EE, all samples were normalized.

Comparative diversity patterns emerged between TOT and IN with both V7 and V4 18 s rDNA β diversity analysis. For both barcodes, the highest number of ASV were found (in both absolute number and percent over the total ASV number) with the IN DNA

Current Biology Report



extraction method, throughout the whole core (Table S2). The highest ASV number (both in absolute number and percent over the total ASV number) were found in the middle section of the core (from 148 to 160 cm) during the Middle Ages (Figure S2C). There were progressive decreases in diversity toward the upper section of the core (more recent times) and the deeper layers (more ancient times) (Figure S2C). Sample assemblages (β diversity, analysis based on the Jaccard diversity index) were similar between TOT and IN. EX samples differed in ASV composition (Figure S2D). Embryophyta (plants) and Unknown ASVs were preferentially amplified in EX samples. BH sediment sample layers could be separated into two groups according to both amplicon sequence variant (ASV) richness and β diversity the first including layers from 2 to 231 cm, and the second composed of deeper core layers, from 233 to 340 cm, with lower ASV richness (Figure S2D). The rarefaction curves for each sample showed different TOT and IN richness saturation according to the core layer depth (Figure S2F). The decrease in ASV diversity and DNA concentration in the lower part of the core was associated with a different sediment granulometry. *Sed*aDNA likely had been better preserved in the silty–muddy sediments of the upper part of the core than in the coarse sediments of the lower part. Given these differences, we could not compare the DNA archives of the lower part of the BH core (233–340 cm) to the more recent ones. Therefore, we performed further analyses of protist diversity using normalized data from the upper part of the core only (3–231 cm), which covered a period of ca. 1400 years (including the Anthropocene period), from the Middle Ages (Vth–VIth centuries) to the contemporary era (XXIth century).

Most of the ASV obtained with the TOT sedaDNA extraction method were common to those obtained with the IN sedaDNA when considering the \geq 1% abundant protist ASVs, with on average 69% and 58% of TOT ASVs common to IN when using the V7 and V4 barcodes, respectively (Figure S2E). These values increased to 76% (V7) and 65% (V4) when considering only ASVs from the surface to 231 cm (Figure S2E). In the lowest part of the core most of the ASVs that are not in common between IN and TOT were only amplified with TOT extraction method. Over the entirety of the core, 83% (V7) and 82% (V4) of these common ASVs were assigned to protist genera known to include species that can produce resting stages (Data S2).

Pollutant analyses

Heavy metal and PCB analyses were carried out on 12 sediment samples of the BH core, covering the period of the industrial revolution of the XIXth century to contemporary times (AD 2003 ± 2). Pollutant analyses were carried out on the same samples used for genetic analyses when enough material was available. Whenever necessary, additional samples of lower or upper sediment layers were exploited. For heavy metals, further control analyses were performed on more ancient sediments. All reagents, labware acid cleaning, and solution dilutions for elemental and isotope analyses were performed using 18.2 MΩ.cm⁻¹ H₂O (Nanop System) and ultra-pure acids (PlasmaPure Plus grade, SCP science). Unsieved dry aliquots of sediments were digested in Teflon bombs on a coated graphite block using a multiple-step acid procedure with hydrofluoric acid, hydrochloric acid, and nitric acid. The final extract solution was split for subsequent elemental and isotope analyses. Procedural blanks and international certified reference materials (MESS-3 from NRC - CNR) were processed in each batch of sediment samples and followed the complete procedure (digestion, elemental analysis and isotope measurements). Elemental concentrations of Ag, Al, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Ni, Pb, V, and Zn were determined by inductively coupled plasma mass spectrometry (Q-ICP-MS, iCAP Q, Thermo Fisher Scientific). The accuracy, indicated by average bias, was always within 10% of the certified values of reference material for the considered elements. The Pb isotope ratio (²⁰⁶Pb/²⁰⁷Pb and ²⁰⁶Pb/²⁰⁸Pb) measurements were performed with the same Q-ICP-MS equipment. Mass bias and instrumental drift were corrected with a standard bracketing method using the NIST SRM-981 standard reference material. The 206 Pb/ 207 Pb and 206 Pb/ 208 Pb internal relative SD averages were 0.15 ± 0.05% and 0.47 ± 0.13% (1 s, n = 20), respectively.

To compare particle results from different locations with variable physical and chemical composition, a normalization procedure is commonly applied. The dilution effects related to grain size and mineralogical heterogeneities were decreased by the normalization of the Al concentration in the sample to the concentration of a reference element. Al was used as the reference element for the particulate material due to (i) its conservative behavior in the core; (ii) its characterization of the clay-size fraction, which is enriched with most trace elements; and (iii) its very low sensitivity to human activities. Normalized metal concentrations were obtained by dividing the concentration of the studied element by the concentration of a normalizer element in the same sample (X in Equation 1). Normalization to Al is widely used as a "proxy" of the clay content, generally representative of the finest and more efficient fraction for element sorption. The reference ([Element]/[X]) represents the local background determined as the concentration ratio considered as free of anthropogenic influence.

Three heavy metal profile groups were identified in the sediment samples analyzed of the BH core. Group I includes Li (Figure 4), V, and Cd (Figure S4A); group II includes Ni (Figure 4) and Cr (Figure S4B); and group III includes Hg (Figure 4), Ag, Co, Cu, Mn, Pb, and Zn (Figure S4C). Elements of the first group were closely related to AI concentrations and the dynamics of the normalized concentrations were constant with mean \pm SD values of 12.9 ± 0.8 mg kg⁻¹ for Li, 19.8 ± 1.0 mg kg⁻¹ for V, and 0.046 ± 0.007 mg kg⁻¹ for Cd (Figures 4 and S4A). The elements of the second group, Cr and Ni, had constant normalized concentrations along the core with values of 13.9 ± 0.6 mg kg⁻¹ and 4.8 ± 0.2 mg kg⁻¹, respectively. There were two peaks of normalized concentrations in the years 1947 ± 11 and 1913 ± 16 with an increase in concentrations up to 50% (20.4 mg kg⁻¹ for Cr (Figure S4B) and 9.8 mg kg⁻¹ for Ni, Figure 4). Elements of the third group had increasing normalized concentrations from 1958 ± 9 to more recent times (surface layers) (Figures 4 and S4C).

The natural background was established using the lowest element/Al concentration ratios in the core (38–39 cm depth). The relative enrichment factor (EF) of trace metals was used as an index to estimate the degree of anthropogenic contamination:

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Current Biology Report

EF = ([Element]sample / [X]sample) / ([Element]reference / [X]reference). (Equation 1)

Many pollution classes have been defined for EF values. Considering the low variability of the local background and following previous class, we assumed: EF values < 1.5 representing no or minimal anthropogenic enrichment; values between 1.5 and 3 representing a moderate anthropogenic signature; values between 3 and 10 indicating a moderately severe anthropogenic contribution; and values > 10 underlining a severe anthropogenic impact. Every trace metal element measured in the core before 1902 \pm 17 had an EF < 1.5, representing minimal anthropogenic enrichment, except for Hg, which showed a higher EF (1.7). The two concentration peaks observed at 1947 \pm 11 for Cr and Ni (Figures 4 and S4B) correspond to a significant anthropogenic impact (EF = 2.0 for Ni). After 1947 \pm 11, the pattern of normalized concentrations and associated EF remained low for some elements. The EF was mainly between 0.9 and 1.5; the relatively low concentrations of Li, V, Co, and Cd over the whole profile demonstrated that these elements are mainly from a lithogenic origin and that there was no significant anthropogenic source for these elements. The EF and concentrations of others trace metals (Ag, Cu, Hg, Pb, and Zn) progressively increased over time, with maximum EF values between 1.9 and 9.5. These values demonstrated a moderate to severe anthropogenic contamination in the order Zn < Pb < Ag < Cu < Hg.

Given that ordinary chemical reactions will not cause variations in the isotopic composition of Pb, it has been demonstrated that Pb is a powerful tracer of sources. To assess the origin of Pb in the sediment of the BH core, we used isotopic compositions as pertinent tools (Figures 4, S4D, and S4E). Normalized Pb concentrations in the sediment core showed constant values ($3.4 \pm 0.1 \text{ mg kg}^{-1}$) from 40.5 to 18.5 cm depth (Figure S4D) with constant $^{206}\text{Pb}/^{207}\text{Pb}$ (0.483 ± 0.001) (Figures 4 and S4D) and $^{206}\text{Pb}/^{208}\text{Pb}$ (1.198 ± 0.002) ratios (Figure S4D). Those ratios could be considered as the natural local background ratios, which is consistent with natural Pb isotope signatures referred to in previous studies performed in France coastal areas ($^{206}\text{Pb}/^{208}\text{Pb}$ ratio (1.190 ± 0.002) (Figure S4D). At 1936 ± 12, a light peak of normalized Pb concentrations is observed correlated with a slight decrease of $^{206}\text{Pb}/^{208}\text{Pb}$ ratio (1.190 ± 0.002) (Figure S4D). At 1936 ± 12, a light peak of $^{206}\text{Pb}/^{207}\text{Pb}$ corresponded to the World War II period (Figure 4). Across more recent times, $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{206}\text{Pb}/^{208}\text{Pb}$ reached a natural local background ratio and normalized Pb concentrations increased more than threefold (Figure S4D). Concomitant variations in Pb concentrations and isotopes ratios showed the importance of anthropogenic activities and inputs in the Pb environmental cycle (Figure S4E).

The solvents (n-hexane, n-pentane, acetone, and iso-octane) used for Polychlorinated biphenyl (PCB) analyses were of trace analysis grade and supplied by SDS (France). PCB analyses were performed as described by Horri et al.⁷¹ and Breivik et al.⁷² Briefly, PCBs were analyzed by pressurized liquid extraction with the SpeedExtractor E-914 (Buchi Lab., Switzerland). The sulfur co-extracted with the organic compounds was removed using a tetrabutylammonium sulfite treatment before sequential purification with concentrated sulfuric acid and by adsorption chromatography on a Florisil column. The purified extracts were analyzed using gas chromatography with electron capture detection (GC-µECD, Agilent 7890B) equipped with two injectors, two columns (CP-Sil 19 and HT08, Chrompack), and two electron capture detectors (µECD Ni63). Among PCBs, the seven indicator PCB congeners were measured (CB28, 52, 101, 118, 138, 153, and 180), their sum being given as Σ 7PCBs. A few other congeners were also analyzed to describe a larger range of chlorination from 3 to 8 chlorine molecules (CB31, 44, 49, 105, 110, 128, 132, 149, 156, 170, 187, and 194) to check the appropriate resolution of the gas chromatography system, and because they can highlight underlying mechanisms acting on the distribution of organic contaminants. Analytical blanks were systematically measured every six samples. The blanks were about 0.2 pg μ l⁻¹, twentyfold less than the concentrations of the lowest standard of CB153 (i.e., < 0.1 ng g⁻¹), and much less for other determinants. Finally, six replicates of a reference material (BCR-SRM1491b) were analyzed to determine the accuracy and precision of the method. PCB recoveries varied between 77% and 115%. Hydrophobic and persistent organic contaminants such as PCBs have a particular affinity for the organic material and differences in the sediment organic fraction can lead to differences in contamination levels. Therefore, to overcome these differences and to compare the different fractions, a standardization procedure was applied and the PCB concentrations were expressed per g of organic carbon in dry sediment.

Historical archive documentation

The departmental archives of Brest were consulted to find data, video, and pictures about the anthropic activities and events that occurred in the Bay of Brest that could have caused any pollution in the harbor area. In particular the, documents were analyzed concerning World War II (Figure S1C) and the Ocean Liberty vessel explosion. Some qualitative (videos, pictures) and quantitative (number of bombs per year, contents of the Ocean Liberty vessel) data were considered for the interpretation of shifts of the paleocommunities.

QUANTIFICATION AND STATISTICAL ANALYSIS

 α (intraspecific) and β (interspecific) diversity and the diversity index were calculated using the vegan R packages Dplyr and Tidyr of R v3.4.4.⁵⁷ α diversity analyses were used to discriminate observed richness patterns in the BH core for all *seda*DNA extraction methods (TOT, IN, and EX) (Table S2). A non-metric multi-dimensional (NMDS) analysis inferred from a dissimilarity matrix based on the Jaccard index was carried out to analyze β diversity among TOT, IN, and EX samples of the BH core (Figure S2D). After the NMDS analysis, the ANOSIM test was performed to identify statistically similar groups of samples. Plots were created using the ggplot2 R package.⁶³

A chronological clustering of the BH, DE, and EE samples was performed to detect the dates and the hierarchical order of modification in the ASV paleocommunity structure. Prior to this analysis, relative abundances of ASV taxonomically assigned to the same





genera/groups were cumulated. This cumulated ASV was named with the genus and/or taxonomic groups to which it belongs (according to PR² annotation). ASV not assigned to any species or genera assigned were cumulated in a group named Unknown. For simplicity, in the Results the taxonomic name of the genus or groups is used to indicate the cumulated ASVs. Separate MRT analyses⁶⁴ were performed on the protist, dinoflagellate, and stramenopile cumulated ASV data from the BH, DE, and EE samples. This time-constrained analysis was performed according to the instructions proposed by Borcard et al.,⁷³ and the size of each tree was selected using the graphs of the relative error. The R package mvpart was used for this analysis. MRT level 1 is shown in Figures 2, 3, and S3.