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SUMMARY EN:

With more than 10% of the global population living at elevations of less than 10 m above sea level, extreme wave events, including storm surges and tsunamis, pose significant hazards to coastal communities and infrastructure around the world. The impact of these events will be further accentuated by anthropogenically-driven sea-level rise, emphasisng the need for better understanding of their potential future frequency and size. To develop evidence-based hazard assessments, we rely on records of the past occurrence of extreme wave events. Whilst such understanding may be founded on instrumental and historical data sources, the recurrence interval between the largest extreme wave events may be far in excess of historical documentation. The analysis of geological records presents a complementary approach, with sedimentary and geomorphic archives providing evidence for the occurrence and characteristics of palaeostorms and palaeotsunamis over multi-millennial timescales.

A range of coastal environments, including tidal marshes, coastal lowlands, lakes and uplifted beach ridge systems, may preserve evidence for past extreme wave events through the presence of laterally extensive sand sheets. The scientific community has developed a wide range of techniques to study these deposits, with approaches incorporating sedimentology, macro- and micropalaeontology, geochemistry and geomorphology. Despite this interest, differentiation between the sedimentary evidence for tsunamis and storms remains a major unresolved issue. Both storm surges and tsunamis may be characterised by wave heights and velocities significantly in excess of normal waves. Both storm surge and tsunami deposits may consequently share features resulting from erosion, transport and redeposition of sediment, including erosive basal contacts, rip-up clasts, upward fining sequences, landward thinning and fining, marine or mixed macro- and microfossil assemblages and increased elemental salinity indicators and heavy minerals.

In the scope of GEN-EX, the overall aim was to pioneer the use of metagenomic approaches to study extreme wave events in different environments and establish their potential for a wide range of future sediment-based geoscience and biological questions. Three objectives contributed to this aim:

1. Quantify the relationship between water depth and the distribution of different species of foraminifera using both classic assemblage methods and metagenomic approaches.

2. Assess the potential for identifying key indicator species in extreme wave deposits (tsunami and storm) in two different climate settings based on both assemblage approaches and metagenomic high-throughput sequencing techniques.

3. Establish how metagenomic approaches contribute to consistent and reliable differentiation between the sedimentary evidence for storms and tsunamis in coastal settings.

To achieve this, tsunami deposits were sampled from two sites: coastal peat sections at three locations on the Shetland Islands, UK, dated to approximately 1.5, 5.5 and 8.15 cal ka BP, respectively, and from Chile. The Chaihuín estuary in southern central Chile has sedimentary deposits of the historical tsunamis of 1737 and 1960.

A regional focus was set on the Shetland Islands, UK, where tsunami deposits are unequivocal and well accessible in the field. The 8.15 cal. ka BP Storegga Tsunami represents the most prominent Holocene tsunami in the Northern Atlantic Ocean affecting the majority of the North Sea and generating run-up

of up to >20 m on Shetland. Furthermore, the Shetland Islands are located in a crucial geographic position for reconstructing palaeo-storminess since cyclones which mostly form near Iceland pass by the Shetlands prior to reach the densely populated coasts of mainland Europe.

The Scottish tsunami deposits were investigated with classic approaches by utilising integrative high-resolution grain-size analysis, CT scanning, multi-sensor core logging and geochemical analyses. When applying classical micropalaeontological techniques, no foraminiferal tests were found in any of the tsunami deposits, whilst inter- to subtidal offshore source deposits show moderate to high foraminiferal concentrations. The diversity of the foraminiferal assemblages increases with water depth inside the fjords. The lack of Foraminifera in the onshore tsunami deposits of obviously shallow marine origin indicates complete post-depositional dissolution of Foraminifera tests and also possible degradation of extra-cellular DNA, most likely due to vertically confining peat layers with very low pH. It could also be that across the time scales of tsunami deposition on Shetland, DNA had already degraded.

During the project, we conducted extensive optimizations of the different steps for metagenomic approaches, including sterile processing of sediment samples, and DNA extractions from sediment samples and individual Foraminifera. For the PCR amplification step, we tested many different PCR primers and conditions separately and in different combinations. The absence of a suitable molecular database for our target Foraminifera (as indicators for extreme wave events) made it next to impossible to design primers based on published DNA sequence data. While we managed to obtain DNA sequence data from some freshly collected Foraminifera from the southern North Sea intertidal, DNA extractions from the Foraminifera in the Scottish offshore transect were mainly unsuccessful, probably because most individuals were already dead at the moment of sampling and therefore did not contain DNA for subsequent genomic approaches. An additional challenge was the presence of episymbionts on some foraminiferal tests, making it impossible to generate DNA sequences from the Foraminifera only.

Furthermore, the DNA sequence data which we obtained indicate that the sediments from Shetland were dominated by non-target organisms (fungi, bacteria, large marine taxa) making it impossible to obtain molecular data from Foraminifera with widely used eDNA approaches.

For the second site in Chile, we managed to successfully obtain a weak molecular signal for Foraminifera from sediments being more than 200 years old, supporting our assumptions that the molecular approach was unsuccessful for the Shetlands because of the sediment chemistry of the deposits and/or their higher age. Due to time and budgetary constraints and the fact that samples from Japan were not available, we mainly focussed on the Shetland Islands, not allowing detailed comparisons between the results on Foraminifera from molecular and morphological analyses for the Chilean samples. Consequently, we could not fully complete objectives 1 and 2 as we could not investigate the distribution of Foraminifera species based on metagenomic approaches in the Shetland Islands and did not have extensive data from the Chilean sites.

Nevertheless, the results from the GEN-EX project are a very significant contribution to the potential application of genomic approaches to tsunami and other sedimentological research. Below, we summarise recommendations for further applications of molecular techniques in tsunami research, thus completing objective 3:

1. Select tsunami sites where sediment conditions are beneficial for preservation of Foraminifera and also for extracellular DNA; these should ideally be situated in cold climates with low oxygen exposure and include sediments with balanced pH and grains to bind the DNA. It also seems that younger deposits have a higher chance to still provide sufficient undegraded DNA for analysis.
2. Fix all sediment samples immediately in liquid nitrogen and keep at -80 °C or fixing them with Lifeguard to stabilize DNA.
3. Conduct also classic approaches in case that the molecular techniques will not be (fully) successful.
4. Use our sterile protocol to process sediment cores and isolate core layers for ancient DNA analyses. Plan sufficient sediment amounts for the molecular approach as low concentrations of Foraminifera (or their DNA) might require multiple extractions from the same core.
5. Use the Norgen Soil DNA isolation or Powersoil kits for DNA extraction from tsunami sediments.
6. When collecting recent Foraminifera to build a reference database, fix them in ethanol and stain them with Rose Bengal to identify living specimens, from which DNA can be extracted. Use the QIAGEN micro extraction kit for DNA extractions from single Foraminifera.
7. For PCR amplifications of living Foraminifera and ancient sediments, add Bovine serum albumin and Dimethyl sulfoxide to the PCR mix and use either the Hot Star Master Mix or the High Fidelity Phusion taq Polymerase. The latter is also suitable for high throughput sequencing. Use the short S14F1 and S15 PCR primers as these also amplify ancient DNA of limited length.
8. When classic DNA barcoding from individual Foraminifera has been successful, use the same primers for large throughput sequencing of sediment cores.
9. Foresee extra deep high-throughput sequencing for eDNA approaches (add additional cost) to accommodate for a high concentration of non-target DNA in the sediment samples requiring deeper sequencing coverage.

Besides these crucial information and experiences regarding the original objectives, the project explored a completely new sedimentary record from the site of Loch Flugarth on the Shetland Island, reflecting the late Holocene variability of storminess and the occurrence of the so-called Dury Voe tsunami, which so far has been considered “uncertain” by some authors.

SUMMARY NL:

Aangezien meer dan 10% van de wereldbevolking op een hoogte van minder dan 10 m boven de zeespiegel woont, vormen extreme vloedgolven, waaronder stormvloeden en tsunami's, een aanzienlijk gevaar voor kustgemeenschappen en infrastructuur over de hele wereld. De gevolgen van deze gebeurtenissen zullen nog worden versterkt door de door de mens veroorzaakte zeespiegelstijging, wat de noodzaak onderstreept van een beter begrip van de mogelijke frequentie en omvang ervan in de toekomst. Om op feiten gebaseerde risico-evaluaties te ontwikkelen, vertrouwen wij op gegevens over het voorkomen van extreme vloedgolven in het verleden. Instrumentale en historische bronnen kunnen hierover meer informatie verlenen, maar het terugkeerinterval tussen de hoogste vloedgolven kan groter zijn dan de periode die gedocumenteerd wordt in de historische bronnen. De analyse van geologische gegevens biedt een aanvullende benadering, waarbij

sedimentaire en geomorfologische archieven bewijsmateriaal leveren voor het voorkomen en de kenmerken van paleostormen en paleotsunami's over een multimilleniumtijdschaal.

In verschillende types van kustomgevingen, waaronder schorren, kustvlakten, kustmeren en strandwallen, is het voorkomen van extensieve laterale zandlagen het bewijs dat er in het verleden extreme vloedgolven plaatsgevonden hebben. De wetenschappelijke gemeenschap heeft een breed scala van technieken ontwikkeld om deze afzettingen te bestuderen, o.a. sedimentologie, macro- en micropaleontologie, geochemie en geomorfologie. Ondanks deze belangstelling blijft het onderscheid tussen de sedimentaire bewijzen voor tsunami's en stormen een belangrijke onopgeloste kwestie. Zowel stormvloeden als tsunami's kunnen worden gekenmerkt door golfhoogten en -snelheden die aanzienlijk hoger liggen dan die van normale golven. Zowel stormvloed- als tsunami-afzettingen kunnen bijgevolg kenmerken gemeen hebben die het gevolg zijn van erosie, transport en herafzetting van sediment, met inbegrip van erosieve basiscontacten, "rip-up"-klasten, "fining upward"-sequenties, landwaartse verdunning en vermindering van de korrelgrootte, mariene of gemengde macro- en microfossielen en toenemende elementaire saliniteitsindicatoren en zware mineralen.

In het kader van GEN-EX was het algemene doel een pioniersrol te vervullen bij het gebruik van metagenomicatoepassingen om extreme vloedgolven in verschillende omgevingen te bestuderen en het potentieel hiervan vast te stellen voor een breed scala van toekomstige geowetenschappelijke en biologische vragen in verband met sedimenten. Drie doelstellingen droegen daartoe bij:

1. De relatie tussen waterdiepte en de verspreiding van verschillende soorten foraminifera kwantificeren met behulp van zowel klassieke assemblagemethoden als metagenomicatoepassingen.

2. Het potentieel beoordelen voor de identificatie van belangrijke indicatorsoorten in extreme-golfafzettingen (tsunami en storm) in twee verschillende klimaatomgevingen op basis van zowel assemblagebenaderingen als metagenomische high-throughput sequencingtechnieken.

3. Vaststellen hoe metagenomicatoepassingen bijdragen tot een consistente en betrouwbare differentiatie tussen de sedimentaire bewijzen voor stormen en tsunami's in kustgebieden.

Om dit te bereiken werden tsunami-afzettingen bemonsterd op twee locaties: veensecties aan de kust op drie locaties op de Shetland-eilanden, UK, gedateerd op respectievelijk ongeveer 1,5, 5,5 en 8,15 cal ka BP, en uit Chili. Het estuarium van Chaihuín in het zuiden van centraal Chili bevat sedimentaire afzettingen van de historische tsunami's van 1737 en 1960.

Een regionale focus werd gelegd op de Shetland-eilanden, UK, waar tsunami-afzettingen ondubbelzinnig zijn en goed toegankelijk in het veld. De 8,15 cal. ka BP Storegga Tsunami is de meest prominente Holocene tsunami in de noordelijke Atlantische Oceaan die het grootste deel van de Noordzee heeft getroffen en op Shetland een opstuwing tot >20 m heeft veroorzaakt. Bovendien bevinden de Shetland-eilanden zich op een cruciale geografische positie voor de reconstructie van paleostormen, aangezien cyclonen die meestal in de buurt van IJsland ontstaan, de Shetland-eilanden passeren voordat ze de dichtbevolkte kusten van het Europese vasteland bereiken.

De Schotse tsunami-afzettingen werden onderzocht met klassieke methodes door gebruik te maken van integratieve hoge-resolutie korrelgrootteanalyse, CT-scanning, multi-sensor core logging en geochemische analyses. Bij toepassing van klassieke micropaleontologische technieken werden er in geen van de tsunami-afzettingen foraminiferen gevonden, terwijl de inter- tot subtidale offshore bronafzettingen matige tot hoge aantallen foraminiferen vertonen. De diversiteit van de foraminiferenassemblages neemt toe met de waterdiepte in de fjorden. Het ontbreken van foraminiferen in de onshore tsunami-afzettingen van duidelijk ondiepe mariene oorsprong wijst op

volledige post-depositionele ontbinding van foraminiferenschalen en ook op mogelijke degradatie van extra-cellulair DNA, waarschijnlijk als gevolg van verticaal ingesloten veenlagen met een zeer lage pH. Het is ook mogelijk dat over de tijdschaal van de tsunami-afzetting op Shetland, het DNA al was afgebroken.

Tijdens het project hebben we de verschillende stappen voor de metagenomicatoepassingen uitgebreid geoptimaliseerd, inclusief de steriele verwerking van sedimentmonsters en DNA-extracties uit sedimentmonsters en individuele foraminiferen. Voor de PCR-amplificatiestap hebben we veel verschillende PCR-primers en -condities afzonderlijk en in verschillende combinaties getest. Het ontbreken van een geschikte moleculaire databank voor onze doelforaminiferen (als indicatoren voor extreme vloedgolven) maakte het vrijwel onmogelijk primers te ontwerpen op basis van gepubliceerde DNA-sequentiegegevens. Terwijl we erin slaagden DNA-sequentiegegevens te verkrijgen van enkele vers verzamelde foraminiferen uit het intergetijdengebied van de zuidelijke Noordzee, waren DNA-extracties van de foraminiferen in het Schotse offshore-transect meestal niet succesvol, waarschijnlijk omdat de meeste individuen al dood waren op het moment van bemonstering en dus geen DNA bevatten voor latere genomicatoepassingen. Een extra uitdaging was de aanwezigheid van episymbionten op sommige foraminiferen, waardoor het onmogelijk was DNA-sequenties te genereren uit alleen de foraminiferen.

Bovendien wijzen de door ons verkregen DNA-sequentiegegevens erop dat de sedimenten van Shetland werden gedomineerd door niet-doelorganismen (schimmels, bacteriën, grote mariene taxa), waardoor het onmogelijk was om moleculaire gegevens van foraminiferen te verkrijgen met de algemeen gebruikte eDNA-toepassingen.

Voor de tweede vindplaats in Chili slaagden wij erin een zwak moleculair signaal te verkrijgen voor foraminiferen uit sedimenten van meer dan 200 jaar oud, hetgeen onze veronderstelling ondersteunt dat de moleculaire benadering voor de Shetlands niet succesvol was vanwege de sedimentchemie van de afzettingen en/of hun hogere ouderdom. Wegens tijds- en budgettaire beperkingen en het feit dat er geen monsters uit Japan beschikbaar waren, hebben wij ons voornamelijk op de Shetland-eilanden geconcentreerd, waardoor de resultaten van de moleculaire en morfologische analyses op foraminiferen voor de Chileense monsters niet in detail konden worden vergeleken. Bijgevolg konden wij de doelstellingen 1 en 2 niet volledig verwezenlijken, aangezien wij de verspreiding van foraminiferensoorten op basis van metagenomicatoepassingen op de Shetland-eilanden niet konden onderzoeken en niet over uitgebreide gegevens van de Chileense sites beschikten.

Niettemin vormen de resultaten van het GEN-EX-project een zeer belangrijke bijdrage tot de potentiële toepassing van genomische benaderingen in tsunami- en ander sedimentologisch onderzoek. Hieronder vatten wij de aanbevelingen voor verdere toepassingen van moleculaire technieken in het tsunami-onderzoek samen, waarmee doelstelling 3 wordt voltooid:

1. Selecteer tsunami-locaties waar de sedimentomstandigheden gunstig zijn voor de conservering van foraminiferen en ook voor extracellulair DNA; idealiter bevinden deze zich in koude klimaten met een lage zuurstofblootstelling en omvatten ze sedimenten met een evenwichtige pH en korrels om het DNA te binden. Het lijkt er ook op dat jongere afzettingen een grotere kans hebben om nog voldoende ongedegradeerd DNA voor analyse te leveren.

2. Fixeer alle sedimentmonsters onmiddellijk in vloeibare stikstof en bewaar ze bij -80 °C of fixeer ze met Lifeguard om het DNA te stabiliseren.

3. Voer ook klassieke benaderingen uit voor het geval dat de moleculaire technieken niet (volledig) succesvol zullen zijn.

4. Gebruik ons steriele protocol om sedimentkernen te verwerken en kernlagen te isoleren voor antieke-DNA-analyses. Plan voldoende hoeveelheden sediment voor de moleculaire aanpak, aangezien voor lage concentraties foraminiferen (of hun DNA) wellicht meerdere extracties uit dezelfde kern nodig zijn.

5. Gebruik de Norgen Soil DNA isolation of Powersoil kits voor DNA-extractie uit tsunami-sedimenten.

6. Wanneer u recente foraminiferen verzamelt om een referentiedatabase op te bouwen, fixeer ze dan in ethanol en kleur ze met Rose Bengal om levende specimens te identificeren, waaruit DNA kan worden geëxtraheerd. Gebruik de QIAGEN micro-extractiekit voor DNA-extracties van afzonderlijke foraminiferen.

7. Voor PCR-amplificaties van levende foraminiferen en oude sedimenten, voeg Bovine serum albumine en Dimethyl sulfoxide toe aan de PCR-mix en gebruik ofwel de Hot Star Master Mix of de High Fidelity Phusion taq Polymerase. De laatste is ook geschikt voor high-throughput sequencing. Gebruik de korte S14F1 en S15 PCR-primers omdat deze ook oud DNA van beperkte lengte amplificeren.

8. Wanneer de klassieke DNA-barcodering van individuele foraminiferen succesvol is geweest, gebruik dan dezelfde primers voor high-throughput sequencing van sedimentkernen.

9. Voorzie extra diepe high-throughput sequencing voor eDNA-benaderingen (extra kosten) om rekening te houden met een hoge concentratie niet-doel DNA in de sedimentmonsters die een diepere sequencingdekking vereisen.

Naast deze cruciale informatie en ervaringen met betrekking tot de oorspronkelijke doelstellingen, heeft het project een volledig nieuw sedimentair archief onderzocht van de site van Loch Flugarth op het eiland Shetland, waarin de laat-Holocene variabiliteit van de stormachtigheid en het optreden van de zogenaamde tsunami van Dury Voe, die tot dusver als "onzeker" werd beschouwd, tot uiting komt.

SUMMARY FR:

Avec plus de 10 % de la population mondiale vivant à une altitude inférieure à 10 m au-dessus du niveau de la mer, les vagues extrêmes, y compris les ondes de tempête et les tsunamis, représentent des risques importants pour les communautés et les infrastructures côtières du monde entier. L'impact de ces événements sera encore accentué par l'élévation du niveau de la mer d'origine anthropique, ce qui souligne la nécessité de mieux comprendre leur fréquence et leur ampleur potentielles à l'avenir. Pour élaborer des évaluations des risques fondées sur des données probantes, nous nous appuyons sur des enregistrements de l'occurrence passée d'événements de vagues extrêmes. Bien qu'une telle compréhension puisse être fondée sur des sources de données instrumentales et historiques, l'intervalle de récurrence entre les événements de vagues extrêmes les plus importants peut être bien supérieur à la documentation historique. L'analyse des archives géologiques présente une approche complémentaire, les archives sédimentaires et géomorphologiques fournissant des preuves de l'occurrence et des caractéristiques des paléo-tempêtes et des paléotsunamis sur des échelles de temps plurimillénaires.

Une série d'environnements côtiers, notamment les marais intertidaux, les basses terres côtières, les lacs et les systèmes de crêtes de plage soulevées, peuvent conserver des traces d'événements de

vagues extrêmes passés grâce à la présence de nappes de sable étendues latéralement. La communauté scientifique a mis au point un large éventail de techniques pour étudier ces dépôts, avec des approches intégrant la sédimentologie, la macro- et la micropaléontologie, la géochimie et la géomorphologie. Malgré cet intérêt, la différenciation entre les preuves sédimentaires des tsunamis et des tempêtes reste une question majeure non résolue. Les ondes de tempête et les tsunamis peuvent se caractériser par des hauteurs et des vitesses de vagues nettement supérieures à celles des vagues normales. Les dépôts d'ondes de tempête et de tsunamis peuvent donc présenter des caractéristiques communes résultant de l'érosion, du transport et de la redéposition des sédiments, notamment des contacts basaux érosifs, des clastes de déchirure, des séquences "upward-fining", un amincissement et un affinement vers l'intérieur des terres, des assemblages de macro- et microfossiles marins ou mixtes et une augmentation des indicateurs de salinité élémentaire et des minéraux lourds.

Dans le cadre de GEN-EX, l'objectif global était d'ouvrir la voie à l'utilisation d'approches métagénomiques pour étudier les événements de vagues extrêmes dans différents environnements et d'établir leur potentiel pour un large éventail de questions géoscientifiques et biologiques futures basées sur les sédiments. Trois objectifs ont contribué à la réalisation de ce but :

1. Quantifier la relation entre la profondeur de l'eau et la distribution de différentes espèces de foraminifères en utilisant à la fois des méthodes d'assemblage classiques et des approches métagénomiques.

2. Évaluer le potentiel d'identification d'espèces indicatrices clés dans les dépôts de vagues extrêmes (tsunami et tempête) dans deux contextes climatiques différents, en se basant à la fois sur des méthodes d'assemblage et sur des techniques de séquençage métagénomique à haut débit.

3. Établir comment les approches métagénomiques contribuent à une différenciation cohérente et fiable entre les preuves sédimentaires des tempêtes et des tsunamis dans les environnements côtiers.

Pour ce faire, des échantillons de dépôts de tsunami ont été prélevés en deux endroits : des sections de tourbe côtière sur trois sites des îles Shetland, UK, datées respectivement d'environ 1.5, 5.5 et 8.15 cal ka BP, et au Chili. L'estuaire de Chaihuín, dans le centre-sud du Chili, contient des dépôts sédimentaires provenant des tsunamis historiques de 1737 et 1960.

L'accent a été mis sur les îles Shetland, UK, où les dépôts de tsunamis sont sans équivoque et bien accessibles sur le terrain. Le tsunami Storegga de 8.15 cal. ka BP est le tsunami le plus important de l'Holocène dans l'océan Atlantique Nord, affectant la majeure partie de la mer du Nord et générant un run-up de plus de 20 m sur les îles Shetland. En outre, les îles Shetland occupent une position géographique cruciale pour la reconstitution des paléo-tempêtes, car les cyclones qui se forment principalement près de l'Islande passent par les Shetland avant d'atteindre les côtes densément peuplées de l'Europe continentale.

Les dépôts de tsunami écossais ont été étudiés selon des approches classiques utilisant l'analyse granulométrique intégrée à haute résolution, la tomodensitométrie, la diagraphie multi-capteurs des carottes et les analyses géochimiques. En appliquant les techniques micropaléontologiques classiques, on ne trouve pas de trace de foraminifère dans aucun des dépôts de tsunami, alors que les dépôts de sources offshore inter- et subtidales présentent des concentrations de foraminifères modérées à élevées. La diversité des assemblages de foraminifères augmente avec la profondeur de l'eau à l'intérieur des fjords. L'absence de foraminifères dans les dépôts terrestres du tsunami d'origine manifestement marine peu profonde indique une dissolution post-dépôt complète des coquilles de foraminifères ainsi qu'une dégradation possible de l'ADN extracellulaire, très probablement due à des

couches de tourbe verticalement confinées avec un pH très faible. Il est également possible que l'ADN se soit déjà dégradé pendant la période de dépôt des tsunamis sur les îles Shetland.

Au cours du projet, nous avons procédé à des optimisations approfondies des différentes étapes des approches métagénomiques, y compris le traitement stérile des échantillons de sédiments et l'extraction de l'ADN des échantillons de sédiments et des foraminifères individuels. Pour l'étape d'amplification PCR, nous avons testé de nombreuses amorces PCR et conditions différentes séparément et dans différentes combinaisons. L'absence d'une base de données moléculaires appropriée pour les foraminifères ciblés (en tant qu'indicateurs de vagues extrêmes) a rendu pratiquement impossible la conception d'amorces basées sur des données de séquences d'ADN publiées. Bien que nous ayons réussi à obtenir des données sur les séquences d'ADN de certains foraminifères fraîchement collectés dans la zone intertidale du sud de la mer du Nord, les extractions d'ADN des foraminifères dans le transect écossais au large ont principalement échoué, probablement parce que la plupart des individus étaient déjà morts au moment de l'échantillonnage et ne contenaient donc pas d'ADN pour des approches génomiques ultérieures. La présence d'épisymbiontes dans les coquilles de foraminifères a constitué un défi supplémentaire, rendant impossible la génération de séquences d'ADN uniquement à partir des seuls foraminifères.

En outre, les données de séquences d'ADN que nous avons obtenues indiquent que les sédiments des îles Shetland étaient dominés par des organismes non ciblés (champignons, bactéries, grands taxons marins), ce qui rendait impossible l'obtention de données moléculaires à partir des foraminifères avec les approches d'ADN électronique couramment utilisées.

Pour le second site au Chili, nous avons réussi à obtenir un faible signal moléculaire pour les Foraminifères à partir de sédiments datant de plus de 200 ans, ce qui confirme nos hypothèses selon lesquelles l'approche moléculaire n'a pas été fructueuse pour les Shetlands en raison de la chimie des sédiments des dépôts et/ou de leur âge plus élevé. En raison de contraintes de temps et de budget et du fait que les échantillons du Japon n'étaient pas disponibles, nous nous sommes principalement concentrés sur les îles Shetland, ce qui n'a pas permis d'effectuer des comparaisons détaillées entre les résultats des analyses moléculaires et morphologiques des échantillons chiliens sur les foraminifères. Par conséquent, nous n'avons pas pu remplir complètement les objectifs 1 et 2 car nous n'avons pas pu étudier la distribution des espèces de Foraminifères basée sur des approches métagénomiques dans les îles Shetland et nous ne disposions pas de beaucoup de données sur les sites chiliens.

Néanmoins, les résultats du projet GEN-EX constituent une contribution très importante à l'application potentielle des approches génomiques à la recherche sur les tsunamis et autres sédiments. Nous résumons ci-dessous les recommandations relatives à d'autres applications des techniques moléculaires dans la recherche sur les tsunamis, complétant ainsi l'objectif 3 :

1. Choisir des sites de tsunami où les conditions sédimentaires sont favorables à la préservation des foraminifères et de l'ADN extracellulaire ; ces sites devraient idéalement être situés dans des climats froids avec une faible exposition à l'oxygène et comprendre des sédiments avec un pH équilibré et des grains pour lier l'ADN. Il semble également que les gisements plus jeunes aient plus de chances de fournir encore suffisamment d'ADN non dégradé pour l'analyse.

2. Fixer immédiatement tous les échantillons de sédiments dans de l'azote liquide et les conserver à -80 °C ou les fixer avec du Lifeguard pour stabiliser l'ADN.

3. Mener également des approches classiques au cas où les techniques moléculaires ne donneraient pas (pleinement) satisfaction.

4. Utiliser notre protocole stérile pour traiter les carottes de sédiments et isoler les couches de carottes pour les analyses d'ADN ancien. Prévoir des quantités suffisantes de sédiments pour l'approche moléculaire, car de faibles concentrations de foraminifères (ou de leur ADN) pourraient nécessiter des extractions multiples à partir de la même carotte.

5. Utiliser les kits Norgen Soil DNA isolation ou Powersoil pour l'extraction d'ADN à partir de sédiments de tsunami.

6. Lorsque vous collectez des foraminifères récents pour constituer une base de données de référence, fixez-les dans de l'éthanol et colorez-les avec du Rose Bengale pour identifier les spécimens vivants, dont l'ADN peut être extrait. Utilisez le kit de micro-extraction QIAGEN pour extraire l'ADN des foraminifères individuels.

7. Pour les amplifications PCR de foraminifères vivants et de sédiments anciens, ajoutez de l'albumine sérique bovine et du sulfoxyde de diméthyle au mélange PCR et utilisez soit le Hot Star Master Mix, soit la High Fidelity Phusion taq Polymerase. Cette dernière est également adaptée au séquençage à haut débit. Utilisez les amorces PCR courtes S14F1 et S15, car elles amplifient également l'ADN ancien de longueur limitée.

8. Lorsque le code-barres classique de l'ADN des foraminifères individuels a été couronné de succès, utilisez les mêmes amorces pour le séquençage à haut débit des carottes de sédiments.

9. Prévoir un séquençage à haut débit plus profond pour les approches de l'ADN électronique (coût supplémentaire) afin de tenir compte d'une forte concentration d'ADN non ciblé dans les échantillons de sédiments nécessitant une couverture de séquençage plus profonde.

Outre ces informations et expériences cruciales concernant les objectifs initiaux, le projet a exploré un registre sédimentaire entièrement nouveau provenant du site de Loch Flugarth sur l'île des Shetland, reflétant la variabilité des tempêtes à la fin de l'Holocène et l'occurrence du tsunami dit de Dury Voe, qui était jusqu'à présent considéré comme "incertain".

Context

With more than 10% of the global population living at elevations of less than 10 m above sea level (https://www.un.org/sustainabledevelopment/wp-content/uploads/2017/05/Ocean-fact-sheet-package.pdf), coastal hazards such as extreme wave events (storm surges and tsunamis) pose significant hazards to coastal communities and infrastructure around the world. The impact of these events will be further accentuated by anthropogenically driven sea-level rise (Rahmstorf, 2017; Arias et al., 2021), emphasizing the need for a better understanding of their potential future frequency and size. To develop evidence-based hazard assessments, we explore historical and geological records of the past occurrence of extreme wave events. While such understanding may be founded on instrumental and historical data sources, the recurrence interval between the largest extreme wave events may be far more than covered by historical documentation. Such long recurrence intervals of events of highest magnitudes can often only be assessed by geological records, which provide evidence for the occurrence and characteristics of palaeostorms and palaeotsunamis over multi-millennial timescales. A range of coastal environments, including tidal marshes, coastal lowlands, lakes, and uplifted beach ridge systems, may preserve evidence for past extreme-wave events through the

presence of laterally extensive sand sheets. The scientific community has developed a wide range of techniques to study these deposits, incorporating sedimentology, macro- and micropalaeontology, geochemistry, and geomorphology. Despite the obvious relevance and interest to society, differentiation between the sedimentary evidence for tsunamis and storms remains challenging. Among some promising sedimentary indicators such as erosive basal contacts, rip-up clasts, upward fining sequences, heavy mineral contents, as well as landward thinning, and fining, microfossil assemblages play a major role. However, in many sedimentary archives of extreme-wave events microfossil remains are either poorly preserved or completely dissolved. The GEN-EX project sought to address the issue of identifying and reconstructing the microfossil record in extreme-wave deposits by applying metagenomic techniques to extract their environmental DNA as a key criterion to differentiate between storm and tsunami deposits. Metagenomics, also known metabarcoding, environmental DNA or eDNA, is a field of biology analysing extracted DNA of communities directly from the environment. While metagenomics has successfully answered a range of questions concerning the biodiversity of current and ancient flora and fauna, it constitutes a novel approach for the extreme wave event community.

Objectives

The overall aim of the research project is to pioneer the use of metagenomic approaches to study extreme wave events in different environments and establish their potential for a wide range of future sediment-based geoscience and biological questions.

1. Quantify the relationship between water depth and the distribution of different species of foraminifera using both classic assemblage methods and metagenomic approaches.
2. 2. Assess the potential for identifying key indicator species in extreme wave deposits (tsunami and storm) in two different climate settings based on both assemblage approaches and metagenomic high-throughput sequencing techniques.
3. 3. Establish how metagenomic approaches contribute to consistent and reliable differentiation between the sedimentary evidence for storms and tsunamis in coastal settings.

Conclusions

GEN-EX aimed to use classical micropalaeontological and molecular approaches to identify foraminifera assemblages (key bioindicators in tsunami and storm deposits), and to unravel their cryptic diversities in onshore extreme wave deposits from their environmental DNA (eDNA) signature. Tsunami deposits were identified was by utilising a multi-poxy approach, involving high-resolution grain-size analysis, CT scanning, multi-sensor core logging and geochemical analyses. By applying classical micropalaeontological techniques, no foraminiferal tests were found in any of the tsunami deposits analysed to date, whilst inter- to subtidal offshore source deposits show moderate to high foraminiferal concentrations, indicating possible severe post-depositional dissolution of foraminifera in the onshore tsunami deposits, which were bracketed in between massive dystrophic peats.

The molecular work conducted in the scope of GEN-EX has shed light on the metagenomic approach to tsunami research via extensive optimizations of the laboratory protocols. In the scope of GEN-EX, we were able to extensively adapt polymerase chain reaction protocols for differing sample styles. We obtained sequences from individual Foraminifera and also amplified foraminiferal DNA from a 1737

tsunami deposit sampled in Chile. We have contributed important data as to which foraminiferal taxa are present in the source waters of Shetland, and which should therefore be targeted species-specific primers in future research.

In terms of assisting hazard assessment, the c. 1500 BP tsunami has been verified by this study, the existence of which had been previously contested, and its chronology has been refined to c. 1400 cal. A BP. Finally, Loch Flugarth has proven to be a very valuable sedimentary archive for storminess and tsunami deposits.

Keywords

Tsunami, storm deposits, metagenomics, Foraminifera, extreme-wave events

1. INTRODUCTION

Onshore tsunami deposits provide crucial information on tsunami recurrence patterns and help to assess the long-term patterns of coastal hazards (Goff et al., 2012; Weiss and Bourgeois, 2012; Engel et al., 2016). Their texture and composition mainly reflect the sedimentary source environments of the local coastal system, as well as bathymetry, onshore topography, wave characteristics and onshore flow conditions. Microfossils (e.g., foraminifers, ostracods, diatoms) are often used to identify tsunami deposits and separate them from other coastal and onshore facies (Mamo et al., 2009; Pilarczyk et al., 2014). Foraminifera within tsunami deposits mostly comprise mixed allochthonous associations dominated by benthic intertidal to inner shelf taxa. Few specimens may also originate from outer shelf to bathyal environments, and even planktonic forms may occur. Furthermore, changes in test numbers, taphonomy, size or adult/juvenile ratios compared to background sedimentation are common. However, post-depositional degradation (e.g., chemical dissolution, bacterial degradation) of carbonate tests often prevents identification, erasing their value as a proxy (e.g., Uchida et al., 2010; Yawsangratt et al., 2012; Pilarczyk et al., 2014; Engel et al., 2016; Hawkes, 2020). This Pioneer Project aimed at developing high-throughput, metagenomic sequencing techniques to identify foraminiferal assemblages in onshore extreme wave deposits from their environmental DNA (eDNA) signature and, thus, make use of this proxy even in case of degradation of the carbonate tests. A regional focus was set on the Shetland Islands, UK, where tsunami deposits are unequivocal and well accessible in the field, dated to approximately 1.5, 5.5 and 8.15 cal. ka BP, respectively. The 8.15 cal. ka Storegga Tsunami represents the most prominent Holocene tsunami in the Northern Atlantic Ocean affecting the majority of the North Sea and generating run-up of up to >20 m on Shetland (Bondevik et al., 2003, 2005b). Furthermore, the Shetland Islands are located in a crucial geographic position for reconstructing palaeo-storminess since cyclones which mostly form near Iceland pass by the Shetlands prior to reach the densely populated coasts of mainland Europe. Even though the only previous studies of palaeo-storminess so far are based on dating cliff-top storm deposits, which are quarried by exceptional waves and transported onshore during major storm events (Hansom and Hall, 2009), fine-grained storm deposits can be expected in the numerous coastal sedimentary archives of the Shetlands archipelago, too. The original plan to also integrate samples from different areas, such as subtropical Japan, the Outer Hebrides of Scotland and Central Chile had to be narrowed down to samples from Chauhuín, Central Chile. Instead, we integrated samples from the intertidal Wadden Sea near the island of Spiekeroog, Germany, to generate DNA sequences from living Foraminifera.

2. METHODOLOGY

2.1 Fieldwork

Chaihuín Chile

Samples from Central Chile were collected within the framework of predecessor projects directed by collaborator Dr. Ed Garrett in 2017. The Chaihuín estuary in southern Central Chile (Fig. 1) reveals sedimentary deposits of the historical tsunamis of 1737 and 1960 (Hocking et al., 2021). Samples were taken either from the surface, from marsh front exposures or using a Russian chamber corer. Further details about the sampling site and the historical tsunami deposits can be found in Aedo et al. (2021) and Hocking et al. (2021).

Fig. 1. Overview of the Chaihuin estuary with location of samples used in this study (base maps: Bing maps). The size of the yellow box on the insert map indicating the position of the large-scale map is exaggerated for better visibility.

Shetland Islands (14–30 March, 2018)

Field work in the Shetland Islands (Fig. 2a) comprised a reconnaissance survey covering the known palaeotsunami sites of Dury Voe, Garth Loch, Benston, Maggie Kettle's Loch (Sullom Voe) (Bondevik et al., 2005b) and Scatsta (Sullom Voe) (Smith, 1993) (Fig. 2b). Permissions to take samples at these sites were obtained from landowners.

At Dury Voe (Fig. 2b), exposures created by a cutbank of the main creek entering the fjord of Dury Voe and by coastal erosion show a thin (1-5 cm) layer of sand bracketed by dystrophic peat laid down by a tsunami ~1500 years ago (Bondevik et al., 2005b). The exposures were documented at eight locations (DV01-DV08) and sampled at two locations using short push cores (11 cores at DV01 and 13 cores at DV08) (Fig. 3).

Furthermore, two samples were taken at DV08 for optically luminescence dating (OSL). Core DV03-RP was taken close to the creek near the coast using a Russian chamber corer (0-290 cm below surface [b.s.]) and a thin gouge corer (290-395 cm b.s.). Furthermore, modern surface samples were taken from the intertidal down to a water depth of 1.1 m inside the fjord (DVM01-DVM04). For analysis of the potential source material, a boat was chartered at Vidlin marina to take subaquatic samples at Dury Voe to get insights into the source material of the onshore tsunami deposits and especially the foraminiferal assemblages at different water depths. Seven samples were taken in total down to a depth of 42.5 m using an Ekman-Birge grab sampler operated in combination with an automatic winch

(Fig. 4). At Aith Voe, southern Mainland, a second boat was chartered for another set of grab samples of the shallow marine surface. Eight samples were retrieved at water depths between 2.5 and 50 m.

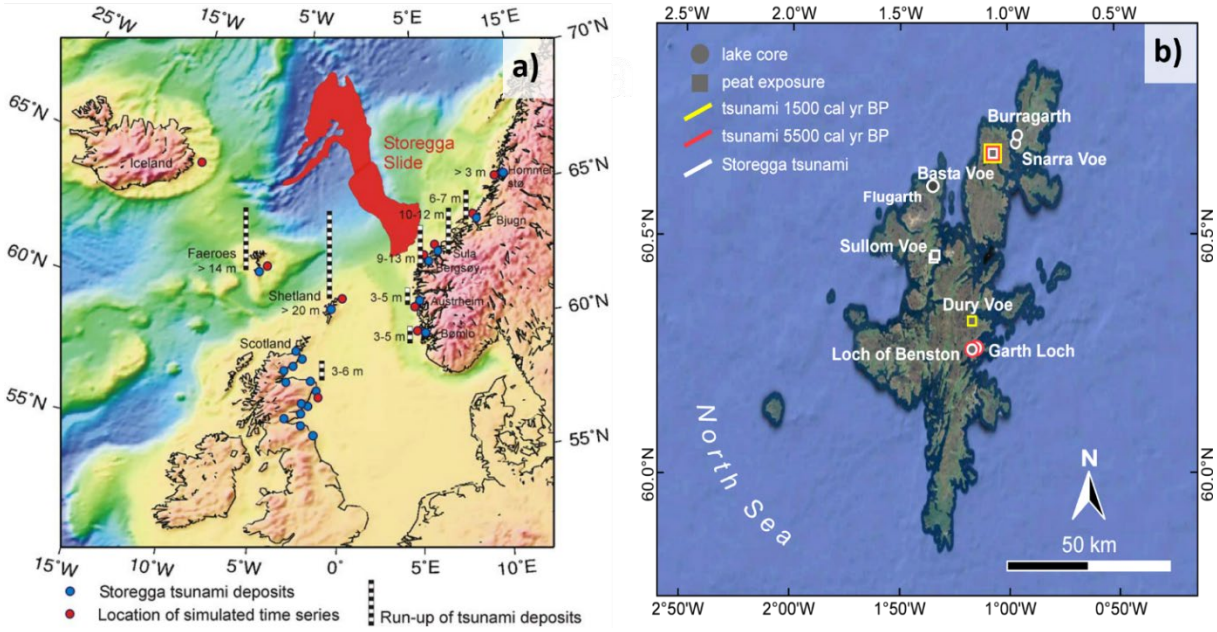
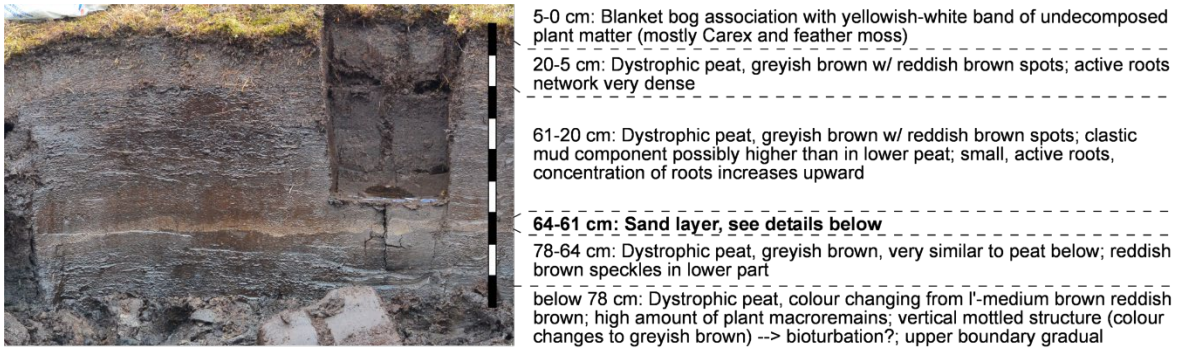


Fig. 2. Sampling sites described in this report: Dury Voe, Flugarth and Maggie Kettle's Loch (Sullom Voe). a) Location of the main Storegga slide as well as correlating debris fans (red area) and tsunami deposits (Bondevik et al., 2005a). b) Field sites on Shetland with details on tsunami deposit occurrence. Evidence indicates three major tsunami events ~8150 (Storegga), ~5500 and ~1500 cal. a BP based on work by Bondevik et al. (2005b).



64-61 cm: Sand layer, yellowish to l'brown, "darkening-upward" through increased mud content, also fining-up; erosive lower boundary, slightly wavy; upper boundary much clearer than in DV01-05; layer continuous;sand coarser than in DV01-05; vertical bioturbation structures; small mud intraclasts?;

Fig. 3. Sediment profile DV08 (see Fig. 4).

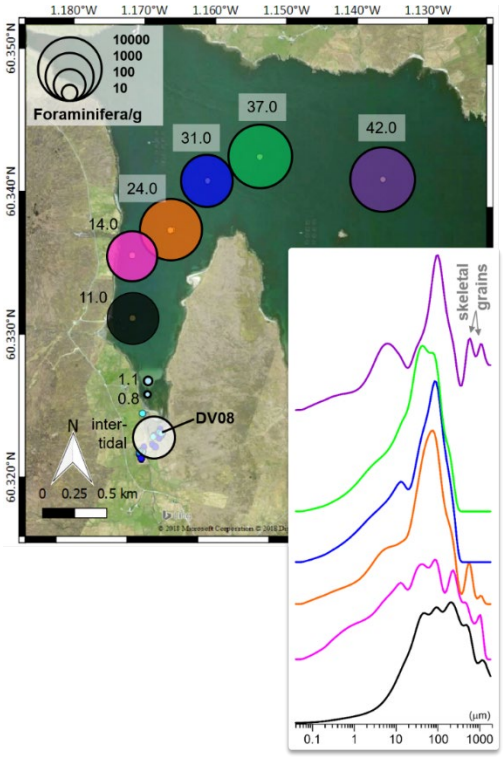


Fig. 4. Sampling sites at Dury Voe with concentration of Foraminifera and particle-size distributions.

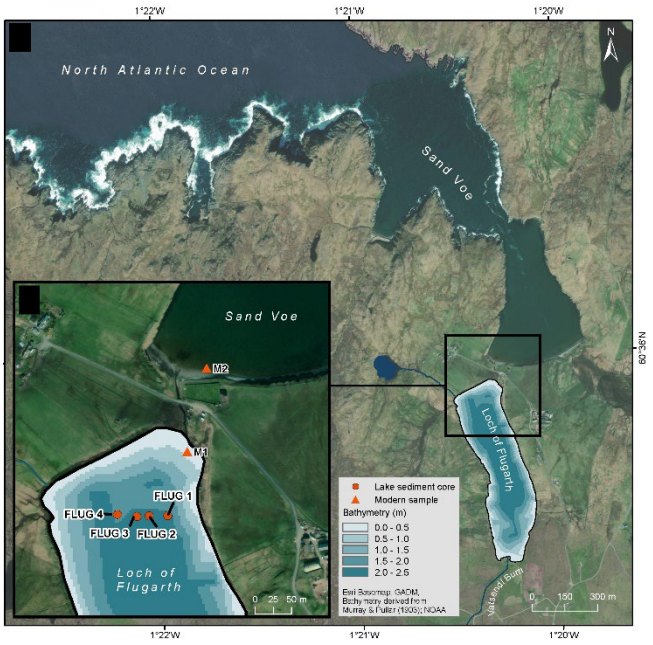


Fig. 5. Overview of Sand Voe bay. The insert shows the location of Loch Flugarth and position of the sediment cores (FLUG 1–4), and the modern environmental samples M1 and M2.

At Garth Loch (Fig. 2b), where sedimentary traces of the 5.5 cal. ka BP and the Storegga tsunamis were identified by Bondevik et al. (2005b), sediment core GAR02 was taken on a distal peat lobe extending into the small loch using a combination of the fat gouge and the Russian chamber corer. Core GAR03 was taken using the fat gouge (0-300 cm b.s.) and the thin gouge corer (300-320 cm b.s.).

At Maggie Kettle's Loch (Fig. 2b), a shallow lagoon separated from the major fjord of Sullom Voe by a sand-to-pebble barrier, the thick Storrega tsunami deposit with several sublayers containing imbricated rip-up clasts was visited. It is described in detail by Bondevik et al. (2003, 2005b). The main site provides an up to c. 30 cm-thick sandy deposit vertically confined by thick dystrophic peat. It is exposed where a small stream cuts into the peat and debouches into the lagoon. The orientation of the exposure is ESE-WNW, c. 128° deviation from N. The deposit thins inland and is traceable up to 9 m above present sea level (Bondevik et al., 2003). Seven short cores were taken comprising the tsunami deposit, as well as two optically stimulated luminescence (OSL) samples and one modern surface sample from the lagoon.

At Loch Flugarth sediment cores were taken from a zodiac using a UWITEC gravity corer and a Russian chamber corer. Loch Flugarth is a small fjord with a shore-perpendicular main axis, which is cut off from the sheltered marine embayment of Sand Voe by a broad barrier of sand, pebbles and small boulders. Sampling localities were chosen from central (i.e., deeper) parts of the lake with sufficient distance to the shoreline to avoid disturbance by wind waves or from erosion at the margins. Three short sediment cores (FLUG 2–4) were taken directly from the lake bottom down to depths of up to 91.7 cm (Fig. 5). The Russian chamber corer (mounted on a manual Edelman coring system) permitted the penetration of a set-up with 4 m of extension rods Russian Peat Corer (RPC) at a water depth of c.

2 m. Based on these assumptions, this short core of 50 cm (FLUG01) reaches a depth of 200-250 cm below lake bottom.

2.2 Sedimentological and micropalaeontological lab work

Samples from Dury Voe (onshore and offshore) and Maggie Kettle's Loch were analysed for grain-size distribution using digital image analysis (Retsch Camsizer P4, particle size range 30–30,000 µm). Loss-on-ignition was measured for selected samples after Heiri et al. (2001). All samples from Chaihuin (Chile), all modern offshore and intertidal samples from Shetland and those from the tsunami deposits at Dury Voe, were investigated for foraminiferal content. Samples were wet-sieved at 63 µm and 100 µm and picked under a binocular microscope (n=25). Samples were picked until 300 specimens were counted. All foraminiferal taxa of these samples were determined at least to genus level, and determinations were supported and confirmed by Dr. Anna Pint, now Institute of Earth Sciences, University of Jena. Taxa determination was also supported by scanning electron microscope (SEM) imaging, carried out at RBINS.

All cores from Maggie Kettle's, Dury Voe and Loch Flugarth were analysed using Ghent University's Geotek multi-sensor core logger (at the Renard Centre of Marine Geology) that measures γ-ray attenuation density, magnetic susceptibility, and spectrophotometric colour. Furthermore, high-resolution line-scan images were taken. At Ghent University Hospital, all cores were subjected to medical CT-scanning to provide pseudo-3D analysis of the stratigraphies. Based on the very promising stratigraphy of Loch Flugarth and the yet unknown record, further proxy data were generated. These comprise high-resolution grain-size distributions using a Fritsch Analysette 22 NeXT Nano, total organic carbon (TOC) using an elementar soliTOC cube at University of Heidelberg's Laboratory of Geomorphology and Geoecology, and XRF data using an Avaatech (GEN-4) X-ray fluorescence core scanner at University of Heidelberg's Institute of Earth Sciences. Thin sections were produced for microfacies analysis. An age-depth model for the stratigraphy of Loch Flugarth was established using ^{14}C and ^{137}Cs data as well as Bayesian age-depth modelling (rBacon; Blaauw and Christen, 2011).

3. RESULTS – SEDIMENTOLOGY AND MICROPALAEONTOLOGY

3.1. Sedimentology and foraminiferal content of modern sedimentary environments and palaeotsunami deposits

Modern offshore sediments from Aith Voe and Dury Voe are mostly very coarse. At several sites, the grab sampling was unsuccessful, as there was almost no sediment covering the bedrock. At Dury Voe, we identified a trend from very poorly (very shallow subtidal) to poorly (intertidal) and moderately (deeper subtidal) sorted. Siliciclastic sands dominate, accompanied by mud and shell debris in the subtidal (Fig. 4). Foraminiferal concentrations at Dury Voe range from low/moderate (intertidal) to high (subtidal), while diversity ranges from two taxa in the intertidal (*Miliammina fusca, Elphidium williamsoni*) to almost 60 in the four deepest samples (dominated by C*ibicides lobatulus, Egerella scabra*) (Figs. 6,7). The entire range of agglutinated and calcareous foraminifera in context of their water depth is shown in Fig. 6. The onshore tsunami deposit at Dury Voe, dated to c. 1500 years ago (Bondevik et al., 2005b), however, consists of fine and medium sand, with a small mud component in the upper part reflected by a decrease in density towards the top of the deposit (Fig. 8). Foraminifera

or any other skeletal grains are absent (Fig. 6). Differences in grain size can be explained by hydraulic sorting of the heterogeneous source sediment inside the fjord, which is also evident from landward fining at this site (Bondevik et al., 2005b).

The tsunami deposit at Maggie Kettle's Loch was also void of Foraminifera, even though, in contrast to the tsunami deposit at Dury Voe, there is a moderate concentration of $CaCO_3$ of up to 2%. Therefore, while there is an abundance of benthic foraminifera present in the shallow marine sediments inside the fjords that represent the source areas for onshore tsunami deposition, the resulting tsunami deposits are void of any foraminiferal test. This is most likely due to excessive carbonate dissolution, as the thin onshore tsunami deposits are bracketed within dystrophic peats, which cover large parts of the Shetland Islands in a thickness of decimetres up to >2 m (Dry and Robertson, 1982). Post-depositional foraminiferal dissolution in tsunami deposits can happen very quickly within only a few years to decades, as e.g. observed after the Indian Ocean Tsunami 2004 (Yawsangratt et al., 2012). Thus, our results indicate the existence of Foraminifera in the source deposits and complete post-sedimentary dissolution – an suitable situation where to test techniques of extracting foraminiferal DNA from the deposit to reconstruct foraminiferal assemblages.

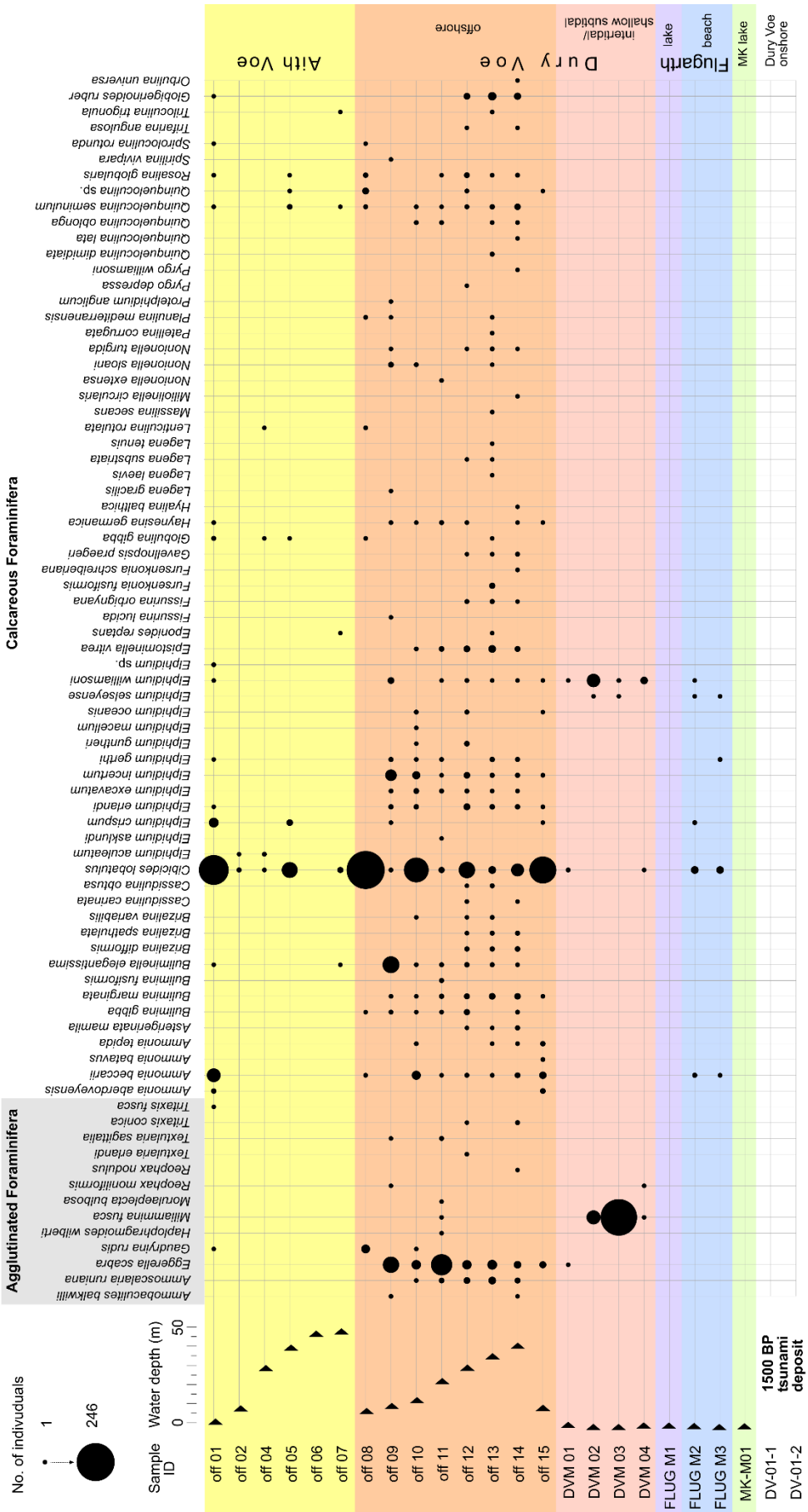

Calcareous Foraminifera
Agglutinated Foraminifera
Ammobaculites balkwilli
Ammoscalaria runiana
Eggerella scabra
Gaudryina rudis
Haplophragmoides wilberti
Miliammina fusca
Morulaepleta bulbosa
Reophax moniliformis
Reophax nodulus
Textularia earlandi
Textularia sagittalia
Tritaxis conica
Tritaxis fusca
Ammonia aberdoveyensis
Ammonia beccarii
Ammonia batavus
Ammonia tepida
Asterigerinata mamilla
Bulimina gibba
Bulimina marginata
Bulimina fusiformis
Bulimina elegantissima
Brizalina difformis
Brizalina spathulata
Brizalina variabilis
Cassidulina carinata
Cassidulina obtusa
Cibicides lobatulus
Elphidium asklundi
Elphidium crispum
Elphidium earlandi
Elphidium excavatum
Elphidium incertum
Elphidium gerthi
Elphidium gunther
Elphidium macellum
Elphidium oceanis
Elphidium selseyense
Elphidium williamsoni
Elphidium sp.
Epistominella vitrea
Eponides reptans
Fissurina lucida
Fissurina orbignyana
Fursenkonia fusiformis
Fursenkonia schreibersiana
Gavelinopsis praegeri
Globulina gibba
Haynesina germanica
Hyalina baltica
Lagena gracilis
Lagena laevis
Lagena substriata
Lagena tenuis
Lenticulina rotulata
Massilina secans
Miliolinella circularis
Nonionella extensa
Nonionella sloani
Nonionella turgida
Patellina corrugata
Planulina mediterranensis
Protelphidium anglicum
Pyrgo depressa
Pyrgo williamsoni
Quinqueloculina dimidiata
Quinqueloculina lata
Quinqueloculina oblonga
Quinqueloculina seminulum
Quinqueloculina sp.
Rosalina globularis
Spirillina vivipara
Spiroloculina rotunda
Triloculina angulosa
Triloculina trigonula
Globigerinoides ruber
Orbulina universa
Aith Voe
Dury Voe
offshore
intertidal/
shallow subtidal
lake
beach
Flugarth
MK lake
Dury Voe
onshore
No. of individuals
1
246
Water depth (m)
0
50
Sample ID
off 01
off 02
off 04
off 05
off 06
off 07
off 08
off 09
off 10
off 11
off 12
off 13
off 14
off 15
DVM 01
DVM 02
DVM 03
DVM 04
FLUG M1
FLUG M2
FLUG M3
MK-M01
DV-01-1
DV-01-2
1500 BP
tsunami
deposit

Fig. 6. The foraminiferal record of modern intertidal and offshore sedimentary environments of Shetland, representing the most likely sources of tsunami deposits. Their record is diverse, while the onshore tsunami deposits investigated at Dury Voe (DV-01) is void of any tests. For location of sites, see Fig. 2b.

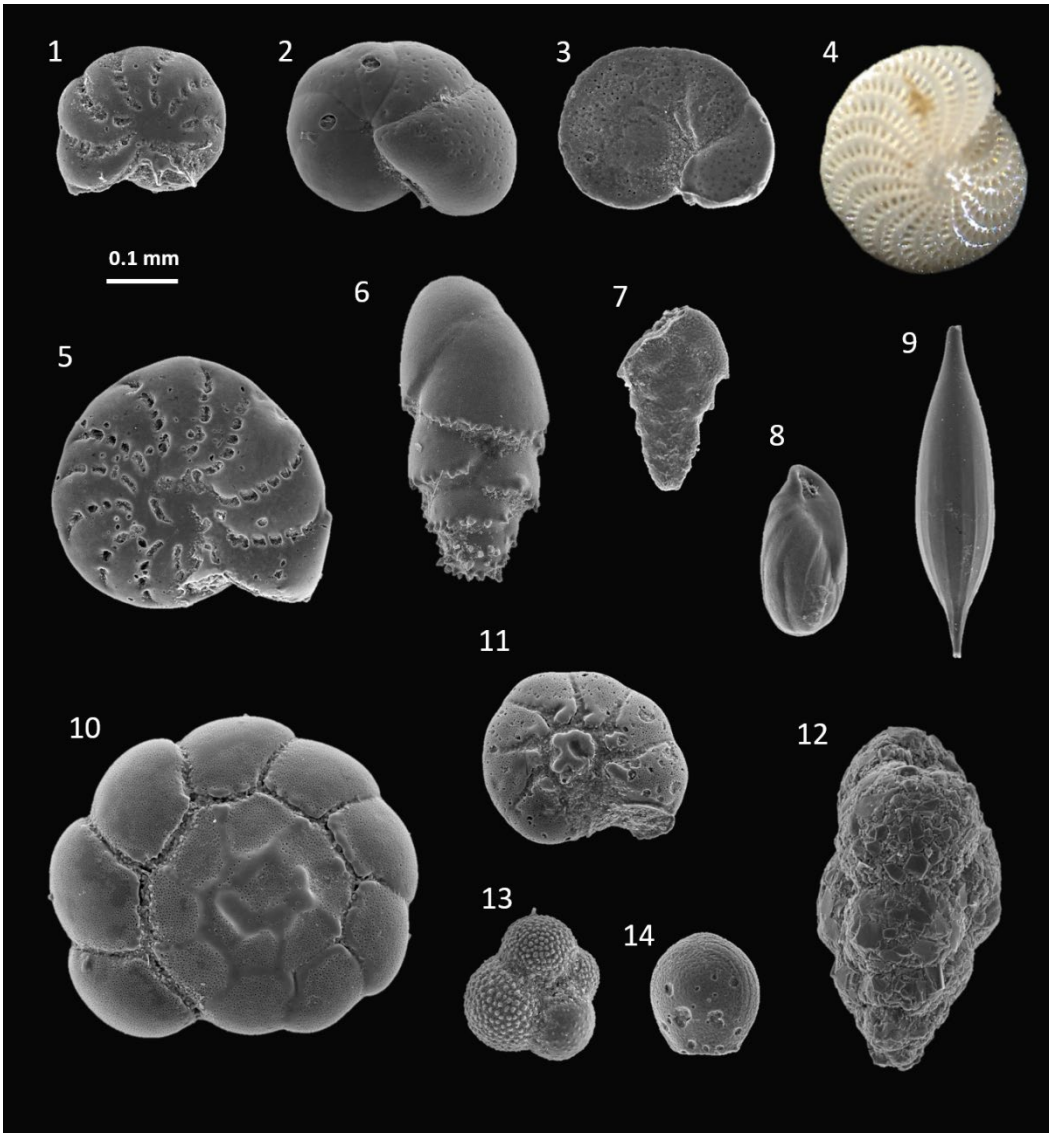


Fig. 7. Scanning electron microscopy images and one light photography image of foraminifera of littoral environments and potential tsunami sediment sources of Shetland, taken from the offshore sediment samples (Fig. 6). 1 – *Haynesina germanica*; 2 – *Cibicides lobatulus* (dorsal); 3 – *C. lobatulus* (ventral); 4 – *Elphidium crispum*; 5 – *Elphidium williamsoni*; 6 – *Bulimina marginata*; 7 – *Bryzalina spathulata*; 8 – *Buliminella elegantissima*; 9 – *Lagena gracilis*; 10 – *Ammonia becarii* (ventral); 11 – *A. becarii* (dorsal); 12 – *Egerella scabra*; 13 – *Globigerinoides ruber*; 14 – *Orbulina universa*.

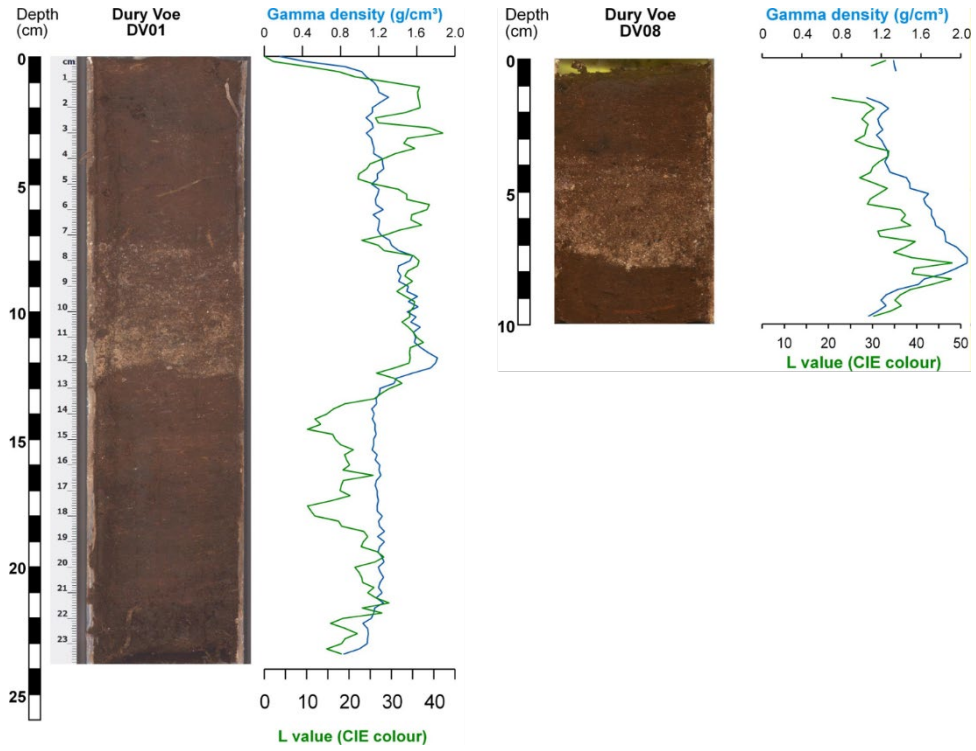


Fig. 8: Gamma density and colour data of the c. 1400 cal. a BP tsunami deposit sampled at two sites at Dury Voe, Shetland (Fig. 4).

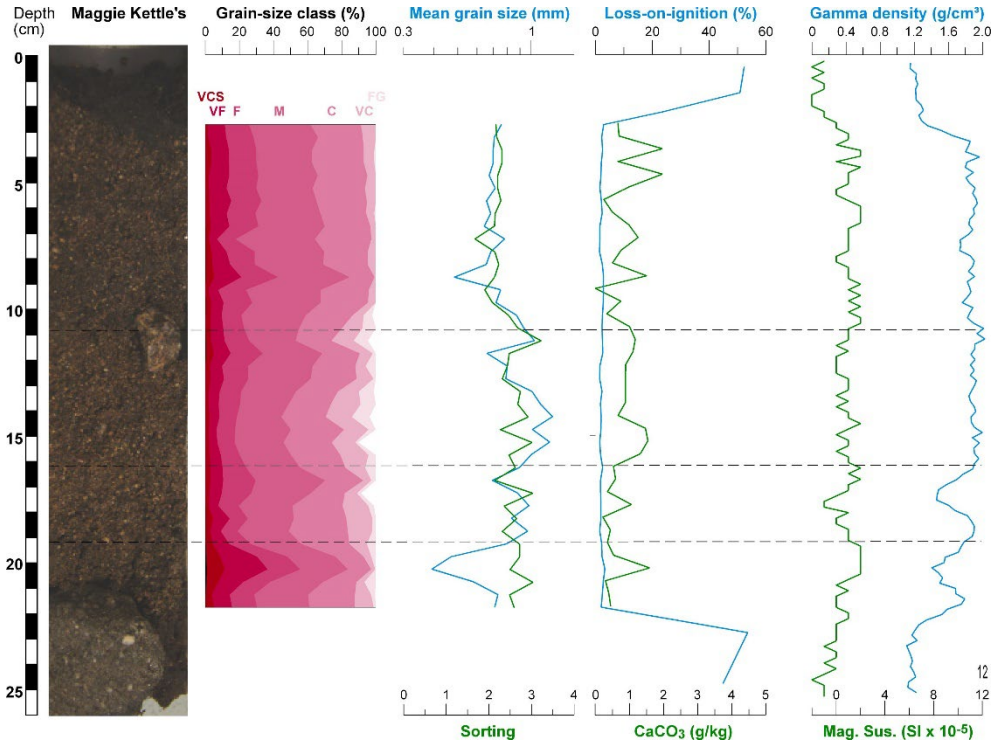


Fig. 9. Storegga tsunami deposit at Maggie Kettle's Loch (Fig. 2b; see also Bondevik et al., 2003, 2005b) with multi-proxy dataset. Grain-size distribution data seems to indicate up to four sedimentation cycles during one tsunami event.

3.2 Exploration of the new sedimentary archive of Loch Flugarth

Loch Flugarth was the only site where a previously unknown sedimentary archive was explored (Fig. 5). The three longer cores show an alternation of organic-rich muds – representing the internal background sedimentation of the shallow lake – and thin sand layers, which are interpreted as the result of marine overwash during extreme storm events. The base of the three longer cores shows a thick sand unit significantly different from the thin sand layers of the overlying stratigraphy, which was interpreted as laid down by a tsunami (Fig. 10).

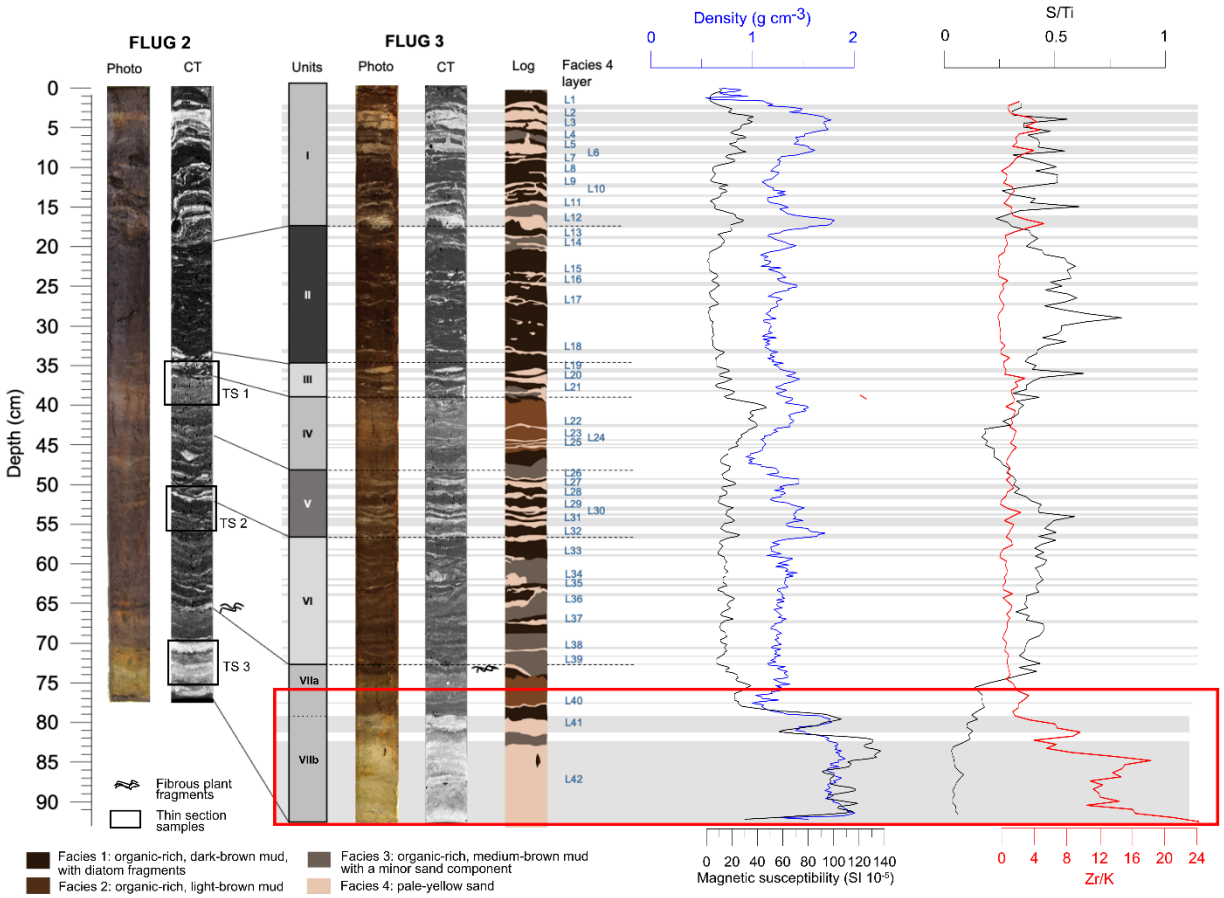


Fig. 10: Overview of the lithostratigraphy. Left: Photograph and CT scan of FLUG 2. Middle: Stratigraphic units, photograph, CT scan and facies distribution of FLUG 3. Right: High-resolution data of bulk density, magnetic susceptibility as well as S/Ti and Zr/K ratios.

3.2.1 Implications for Northeast Atlantic storminess

High-resolution sedimentary records in coastal environments can provide clues on the variability of the intensity and the frequency of storms. Such records of past storminess are required to assess (i) whether storm activity has increased in recent decades and (ii) which future risks may arise from storm surges. The present palaeotempestological study from Loch Flugarth uses the vertical distribution of the 40 thin sand layers as a storm indicator and explores the variability of the underlying climatological processes. Besides the Bayesian age-depth model, which indicates a chronostratigraphy dating back until c. 1400 cal. a BP, this multi-proxy study utilises sedimentological and geochemical analyses (see methods section), as well as the re-analysis of historical data (cf. Lamb, 1991). Especially Si/Ti ratio and

the unimodal grain-size distribution clearly show that the sand layers originate from the beach and the uppermost subtidal and thus are likely representative of storms originating from a northwesterly direction. The data identifies phases of higher storminess around 980–1050, 1150–1300, 1450–1550, 1820–1900 and 1950–2000 cal. a CE. However, a clear correlation of individual sediment layers to single historical storm events is impossible due to the high number of historically documented storms.

Phases of higher storminess correlate with phases of a positive mode of the North Atlantic Oscillation (NAO) (cf. Trouet et al., 2009) and enhanced inflow of North Atlantic water into the Norwegian Sea (Giraudeau et al., 2010) (Fig. 11). The number and thickness of storm layers dated to the Medieval Warm Period (MWP; 950-1350 CE), particularly 980–1050 and 1150–1300 cal. a CE, as well as during the second half of the 20th century, suggests similar storm conditions. The lake sediments reflect storm tracks running north of the Shetland Islands and storms originating from northwesterly to northerly direction. Additionally, a shift of storm tracks towards the northeast Atlantic during warmer periods is assumed.

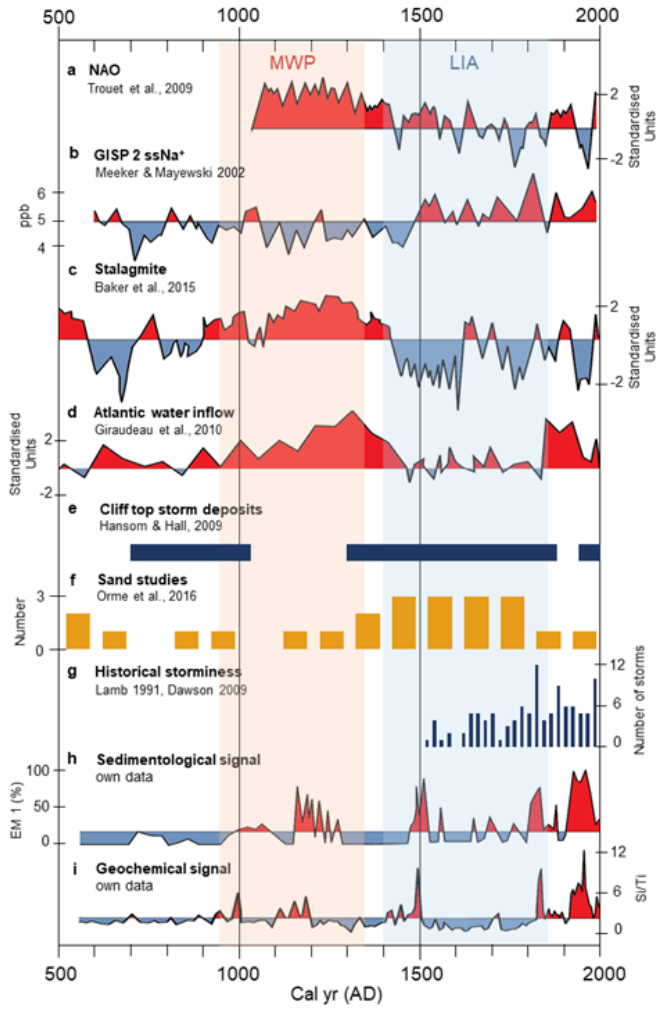


Fig. 11. Comparison between NAO, regional storm records and own data (modified after Orme et al., 2016). a) Winter NAO reconstruction (Trouet et al., 2009). b) Smoothed Greenland sea salt influence (ssNa+) (Meeker & Mayewski, 2002). c) Stalagmite proxy from a Scottish cave (Baker et al., 2015). d) Reconstruction of Atlantic water inflow into the Norwegian Sea derived from concentrations of coccolith Gephyrocapsa muellerae *(Giraudeau et al., 2010). e) Cliff-top storm deposits on the Shetland Islands (Hansom and Hall, 2009). f) Number of studies reporting sand transport in Scotland and Northern Ireland (Orme et al., 2016). g) Number of storms per 20 years from historical documents for northern Scotland and northern North Sea (Lamb, 1991). h) This study: Sedimentological signal of storminess by Endmember 1 from endmember modelling analysis of grain-size distributions. i) This study: Geochemical signal represented by the Si/Ti ratio. For h) and i): Red peaks indicate higher storminess.*

The Little Ice Age (LIA; 1400-1850 CE) had a different wind regime characterised by more northeasterly to southwesterly storms. Storm tracks were shifted further south, resulting in higher storminess in the more southern parts of Europe. Storminess shifted both spatially and temporally with more storms in

spring and autumn rather than in winter, as during the MWP. Winters probably were drier during the LIA due to anticyclonic weather patterns over the North Atlantic.

The higher number and thickness of sand layers in the uppermost part of the Flugarth record confirms that storm tracks are again shifting further north after the LIA, as also dindicated in the most recent IPCC report (Seneviratne et al., 2021). The combination of warmer oceans and cold fresh air from north is expected to form intense low-pressure systems in the future, leading to more severe storms from northwest to southeast and thus higher storm surges in the wider study area (see also Goslin and Clemmensen, 2017). The full study can be found in Hess et al. (2023).

3.2.2 Implications for the tsunami history of the North Sea basin

While onshore deposits of the Storegga tsunami (c. 8150 cal. a BP) are quite abundant on the Shetland Islands, sedimentary evidence of the younger tsunami events c. 5500 and c. 1500 cal. a BP (Bondevik et al., 2005b) is scarce. Sediments of the youngest tsunami (the "Dury Voe" event) have only been found at two sites so far, in the form of thin landward fining and landward thinning sand sheets which are vertically confined by peat (Bondevik et al., 2005b; Dawson et al., 2006). Despite this well-founded evidence, some authors currently consider this event "uncertain" (Long, 2015).

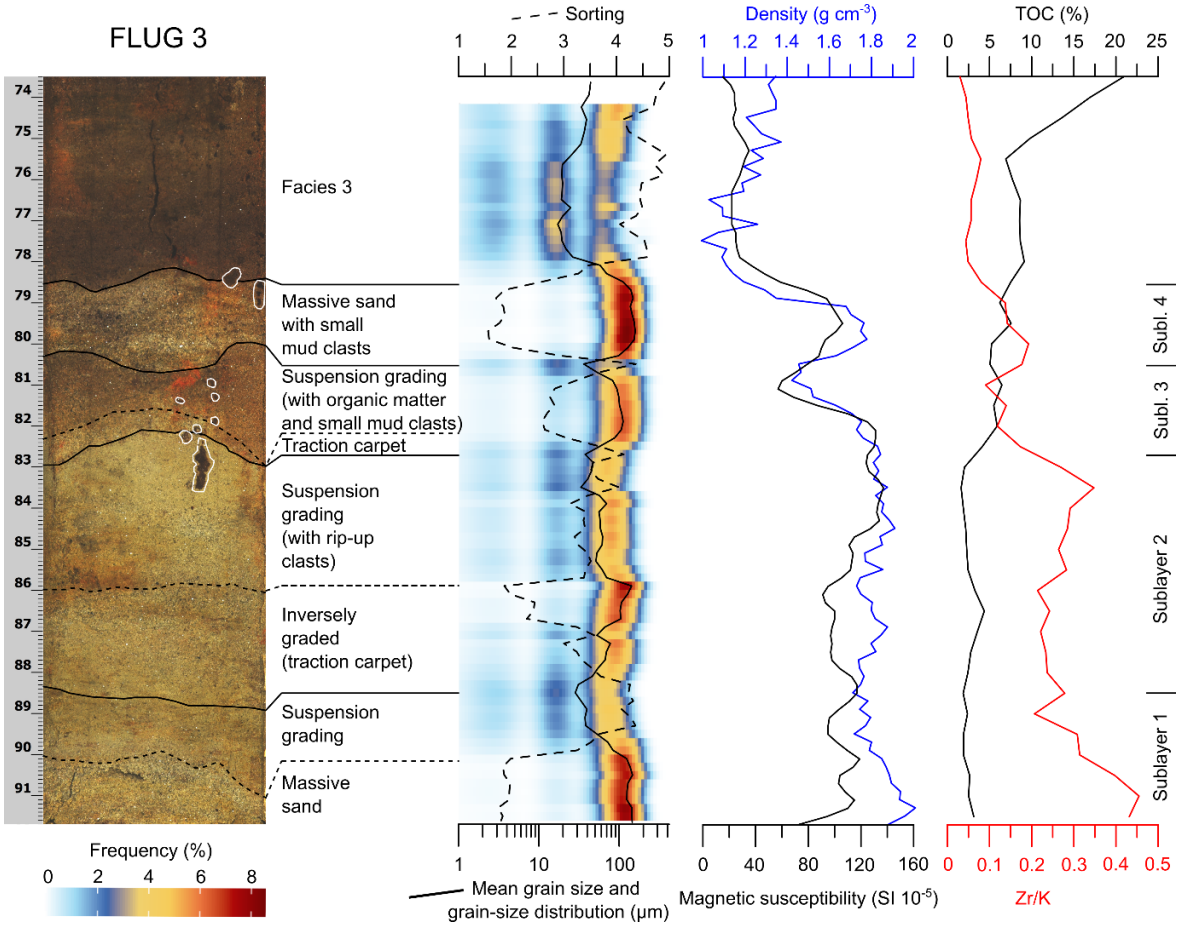


Fig. 12: Core photograph of Unit VII showing the sublayers of Unit VIIb, with small peat clasts marked by thin white polygons. The sublayers are shown in synopsis with mean grain size, sorting, grain-size distribution, magnetic susceptibility, bulk density, TOC and Zr/K.

The basal 13 cm-thick sand unit from the sedimentary record of Loch Flugarth, dated to 426–787 cal. a CE provides additional evidence for the Dury Voe event. High-resolution grain-size analysis identifies four normally graded sublayers with inversely graded traction carpets in the lower part of two sublayers. An organic-rich 'mud' drape and 'mud' cap cover the upper two sublayers, which also contain small rip-up clasts (Fig. 12). Grain-size distributions show a clear difference between the basal sand layer and the coarser and better sorted thin storm layer above. Principal component analysis of the XRF core scanning data also distinguishes both sand units: Zr, Fe and Ti dominate the basal sand, while the thin storm layers are high in K and Si. The enrichment of the basal sand layer in Zr and Ti, in combination with increased magnetic susceptibility, may be related to higher heavy mineral content in the basal sand reflecting the additional marine sediment source of a tsunami deposit below the storm-wave base. The age-depth model and correlation with tsunami deposits of similar age in the Shetland Islands suggests an age around 1400 cal. a BP rather than 1500 cal. a BP as inferred by Bondevik et al. (2005b) and Dawson et al. (2006). Correlation with a historical, allegedly seismic tsunami observed on the Outer Hebrides in 720 CE (Mac Conamhna, 2023) seems questionable based on the chronological evidence. Although the source of the tsunami remains unclear, the lack of evidence for this event outside of the Shetlands suggests that it was smaller than the Storegga tsunami, which affected most of the North Sea basin.

While this study substantiates the existence of the late Holocene Dury Voe tsunami, it prompts the need for (i) further studies on the spatial distribution of its impact through intensified palaeotsunami research in the North Sea region, and (ii) investigations on possible regional source mechanisms that may represent hazards along the coasts of the North Sea at present and in the future. From a more local perspective, this study corroborates the assumption of Tappin et al. (2015, p. 8) that Flugarth "has the potential to preserve tsunami sediments". Loch Flugarth preserves both tsunami and storm deposits that can be unequivocally distinguished based on sedimentological and geochemical criteria. Therefore, we advocate for exploration of the deeper sediment record of Loch Flugarth to improve our chronological estimate of the Dury Voe tsunami and possibly other regional tsunamis such as the Garth tsunami (c. 5500 cal. a BP) (Bondevik et al., 2005b) and extend the palaeotempestological record of the northern North Sea (Hess et al., 2023) deeper into the Holocene. The full study can be found in Engel et al. (2023).

4. RESULTS - METAGENOMIC ANALYSES OF EXTREME WAVE EVENTS FROM DIFFERENT ABIOTIC ENVIRONMENTS

By identifying fragments of DNA in the environment, eDNA approaches present a promising tool for monitoring biodiversity in a cost-effective way. eDNA and metabarcoding approaches originate from classic DNA barcoding (Hebert et al., 2003) where specimens were identified individually with DNA sequence data, mostly of COI, whereas in eDNA techniques, entire communities are analysed at the same time. Such molecular approaches contain several different steps: sampling, DNA extraction, amplification through polymerase chain reaction (PCR), sequencing (for classic DNA barcoding or high-throughput sequencing), and taxonomic identification by comparisons with existing databases. All of these steps require optimisation, and even more so when applying molecular approaches in new research fields and environments or target organisms, which have not been investigated at all or only in pilot studies. This most certainly applies for the GEN-EX project.

In the last years, an increasing number of reviews and recommendation papers have been published describing potential pitfalls and suggesting standards for eDNA and metabarcoding approaches (e.g., Harrison et al., 2019; Zinger et al., 2019; Burian et al., 2021; Rodriguez-Ezpeleta et al., 2021; Gold et al. 2022). These and other publications have also emphasised that the application of eDNA techniques is not trivial (see for example Cordier et al., 2020). A figure in Rodriguez-Ezpeleta et al. (2021) illustrates how the number of publications using eDNA has exploded since 2016. The majority of these eDNA studies have focused on aquatic habitats and analysed water samples using bulk samples of collected organisms or eDNA from the water (Pawłowski et al., 2022). Less papers have been published on eDNA from marine soft sediments (summarised in Pawłowski et al., 2022), with a focus on nematodes, copepods, fungi, viruses and archaea and bacteria; very few have successfully studied Foraminifera as for example from the deep sea (Lejerowicz et al., 2021) and the Arctic (Pawłowska et al., 2020).

Below, we will describe and discuss our approaches and results, following the different steps of the planned metagenomic approach of extreme wave events.

4.1 Field sampling and fixation of samples

4.1.2 Palaeo-tsunami samples (Chaihuín, Chile)

DNA samples were collected by collaborator Dr. Ed Garrett, from Chaihuín, Chile in 2017 (Fig. 1). These samples comprised the 1737 and the 1960 tsunami sand layer, and samples of the bracketing peat layers. Samples were taken with a sterile spatula and stored in sterile falcon tubes until shipment to RBINS.

4.2.2 Modern individuals (Shetland Islands)

Samples for downstream molecular analyses were taken during the offshore transects at Dury Voe (Fig. 4) and Aith Voe. Owing to inconsistent Ekman grab sampling and varied sediment amounts captured during each deployment, it was not feasible to sample triplicates at each of the 15 offshore sampling sites. A total of 31 DNA samples were retrieved during this transect between depths of 2

and 50 m. These individuals were collected to generate individual DNA barcodes and to construct a custom reference database.

4.2.3 Modern inter-tidal sediment (Shetland Islands)

Four modern inter-tidal sediment samples were taken during low tide between depths of -80 and -110 cm below the water level at Dury Voe (Fig. 4). The lagoon at Maggie Kettle's Loch was sampled at c. 40 m into the lagoon, at a water level of -35 cm, and three subsamples were taken from this sample as modern intertidal sediment samples. Samples were taken with a sterile spatula and stored in sterile falcon tubes until shipment to RBINS.

4.2.4 Palaeo-tsunami sediment push cores (Shetland Islands)

A total of six push cores were taken for eDNA at Dury Voe site 01 and another six taken for eDNA from Dury Voe site 08 (Figs. 3,4,8). Two push cores for eDNA were taken at Maggie Kettle's Loch (Fig. 9). Samples were taken with sterile PVC liners of 6.3 cm diameter. Push cores were capped with sterilised caps on both ends, and wrapped in aluminium foil until they were dissected at RBINS.

4.2.5 Sample storage in the field

Samples from all three environment types were kept at 4 °C during the field work and frozen at -20°C in the laboratory until they could be further processed. We had discussed before sampling with experts of the field what the best way of sample storage was, and it had then been recommended to keep them cooled until arrival at the lab; also, because it would have been logistically impossible to freeze the samples in a remote location like the Shetland Islands. Two recent reviews recommend now freezing the samples at -80 °C (Armbrecht et al., 2019; Pawłowski et al., 2022) or fixing them with Lifeguard (Pawłowski et al., 2022) but this kind of information was not available during sampling while GEN-EX was running. Our experience with sampling for the GEN-EX project thus confirms the statement of Pawłowski et al. (2022) that sampling methodologies for eDNA retrieved from sediments is much more challenging than from water samples.

We also later sampled living Foraminifera firstly at Wenduine, Belgium, then secondly at the North Sea coast on the island of Spiekeroog, Wadden Sea, to optimise the PCR protocol further.

4.2 Sample processing

Owing to poor weather conditions at the time of collection, and an unconstrained chronostratigraphy of sediment cores GAR02 and GAR03, palaeo-samples from Garth Loch were not prioritised. For the push core samples collected from the Shetlands, an in-house protocol for the handling and opening of the ancient DNA cores was established at RBINS at the Geological Survey of Belgium. This important resource can be used in future projects. Three cores containing the 1.5 ka deposit from Dury Voe site DV08 (_R1/_R2/_R3), and one core containing the 8.15 ka tsunami deposit from Maggie Kettle's (MK_PC1.1) were opened under stringent clean conditions, photographed and core descriptions logged for classic analyses of the cores. Half of each core was archived, and the other half transported on the same day to a dedicated ancient DNA facility at RBINS. Prior to the cores being subsampled for DNA analyses, the ancient DNA facility was prepared using ultraviolet sterilisation of the work benches (2—3 hours), and SAS-air filtration. Meticulous caution was exercised to sterilise this laboratory prior

to work (e.g., UV and SAS air filtration) and between handling of each sample, especially between localities as recommended by the scientific literature. Both the 1737 and 1960 Chaihuín tsunami deposits were also logged and subsampled along with their bracketing peat layers. For each core ~2.5 g of sediment was subsampled from each layer and divided into three biological replicates.

The samples collected from the Shetland locality Dury Voe during the offshore transect were sieved for Foraminifera using nested sieves of aperture sizes 63 µm and 100 µm, respectively. Morphotype numbers were assigned to each individual, and Foraminifera were photographed under a light microscope. A total of 43 morphotypes were identified from the Shetland modern samples and later determined (Figs. 6,7). Individuals were separated into 1.5 ml Eppendorf tubes and preserved in molecular grade (96%) ethanol for subsequent DNA extraction and PCR amplification.

Foraminifera sampled at Spiekeroog were divided into three subsamples which were taken at each tidal flat site. These were either (i) stained with a Rose Bengal and 100% ethanol (EtOH) solution (to stain the living cytoplasm without affecting downstream molecular analyses), (ii) preserved in 100% molecular grade (EtOH) and iii) a control, stored frozen at -20 °C. A total of nine field samples were taken at Spiekeroog, but our study utilised only the first set of sub-samples, namely the samples that had been stained with Rose Bengal and fixed in ethanol. The sediment was sieved using the protocol described above and individuals of the species *Haynesina germanica* were fixed in 1.5 ml Eppendorf tubes with ethanol, and kept at -20 °C until PCR analyses could be performed at RBINS.

4.3 DNA extractions

The following extraction kits were tested and the manufacturer's protocol modified to develop an optimised in-house protocol: (i) QIAamp DNA Micro Kit (Dury Voe modern individuals), (ii) Qiagen DNeasy Blood & Tissue Kit (Dury Voe and Spiekeroog modern individuals, Dury Voe modern intertidal-sediment) and (iii) the Norgen Soil DNA isolation kit (Dury Voe palaeotsunami, Chaihuín peat, Chaihuín 1737 and 1960 tsunami deposits, Dury Voe modern intertidal sediment).

DNA was extracted from the push core samples using the Soil DNA isolation kit (Norgen) following the manufacturer's protocols as the Powersoil DNA kit (MoBio) was not available to order at the time. aDNA had been successfully extracted in the RBINS facility using the Norgen kit by other research groups. Recently, the Powersoil kit has been recommended by Pawłowski et al. (2022) for eDNA from marine soft sediments while Armbrecht et al. (2019) found a combination of EDTA incubation and bead-beating (as in the Powersoil kit) most effective. These papers were not available at the time when the samples of GEN-EX were processed.

DNA was extracted from individual Foraminifera using the QIAGEN micro extraction kit, measured with the qubit DNA fluorometer and then prepared for Polymerase Chain Reaction (PCR) amplification. From three field replicates during the Chaihuín campaign, a further three biological replicates were prepared for DNA extraction from the samples. A total of 12 DNA samples were extracted for the 1737 tsunami, and three samples were extracted for the 1960 tsunami (as less sand was available for this deposit).

From the Shetland Islands campaign, a total of 18 eDNA subsamples and extractions were processed from palaeo-tsunami core DV08_R1. Another 12 eDNA subsamples and extractions were processed from palaeo-tsunami core DV08_R3, and 12 eDNA subsamples and extractions were processed from

palaeo-tsunami core MK_PC1.1, respectively. Triplicate samples were taken in each described layer (Figs. 4, 13).

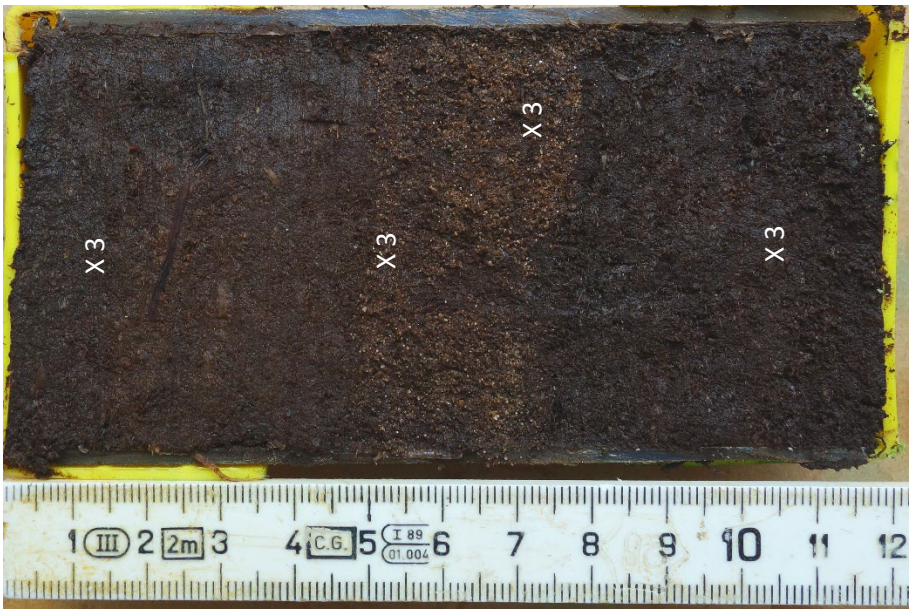


Fig 13: Explanation of the biological triplicates sampled from each of the main described layers in core DV08_R3 (Shetland Islands) (cf. Figs. 3,4,8).

A total of 48 modern individuals from offshore sediment samples 09, 11, 12 and 13 from Dury Voe (Fig. 4) were processed. Owing to time and budget constraints, modern intertidal sediment samples were processed for eDNA extraction only from the Dury Voe locality (i.e., a total of four field samples with three biological replicates each). Finally, 36 individual samples of *H. germanica* were processed from the Spiekeroog campaign; 12 of these extractions were required to further optimise the PCR protocol. This was achieved by staining a set of subsamples collected from the tidal mudflats in Spiekeroog with Rose Bengal (Fig. 14) as only individuals being stained contain cytoplasma and are alive. Individuals which did not uptake the dye were excluded from molecular analyses as they would have likely been degraded.

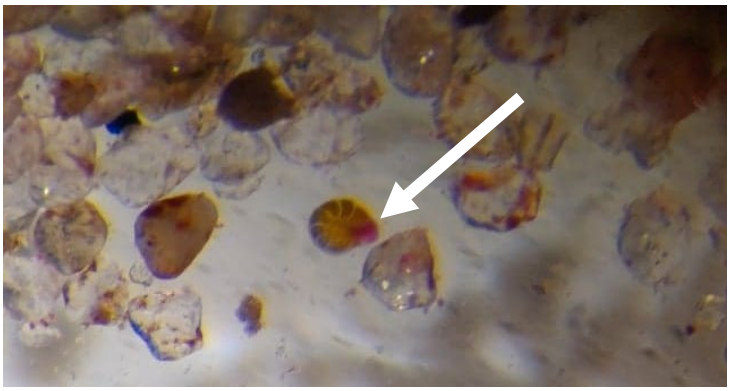


Fig. 14. Rose Bengal-stained single Foraminifera Haynesina germanica *(centre) indicating live at the time of collection.*

4.4 PCR amplifications and Sanger sequencing

In the scope of this project, several different PCR protocols and primers to amplify various genetic regions or genes of Foraminifera were tested. Initially, the nested-PCR protocol by Pawłowski et al. (2002) and Pawłowska et al. (2014) was followed with which we targeted a part of the ribosomal 18S small subunit (Fig. 15).

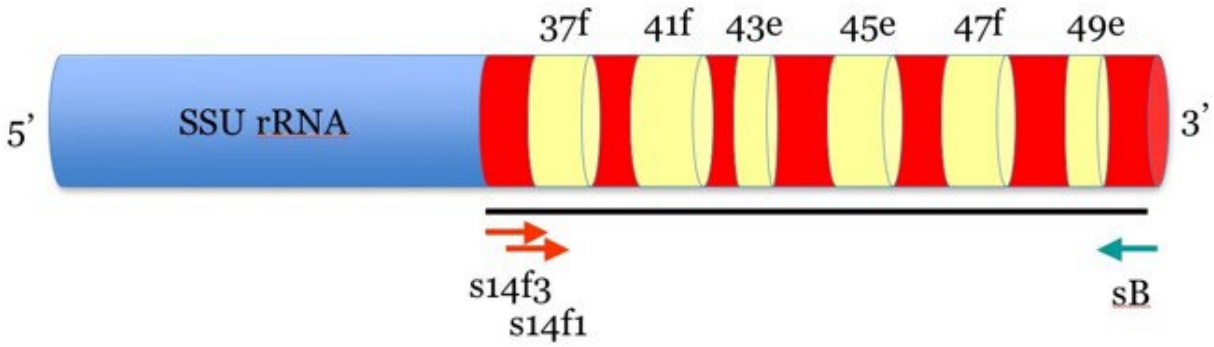


Fig. 15. 18S rRNA PCR showing the targeted 37f–49e regions of the small ribosomal subunit (SSU) (Source: http://forambarcoding.unige.ch/).

The nested PCR protocol using AmpliTaq polymerase was unsuccessful for the samples. Given that many factors contribute to the success of a PCR, we further determined which parts of our protocol (i.e., if the nuclear primers S14F1, F3, S17 and SB or the polymerase) could be the problem. We first tested two other polymerases (i.e., HotStartMM and DreamTaq). As our results were still inconclusive, the AmpliTaq polymerase from the Pawłowski et al. (2002) protocol was also tested together with a positive control, namely another taxonomic group (Amphipoda) where the PCR reaction had previously been successful. The AmpliTaq did not generate any PCR amplicons, also not with the amphipod samples revealing that this polymerase was not suitable.

Another small-scale experiment was then conducted using universal ribosomal and mitochondrial PCR primers, (see Table 1) with other polymerases (HotStartMM and DreamTaq). We successfully obtained PCR amplicons with the LSU primers, but the BLAST results (Altschul et al., 1990) from the DNA sequences obtained by classic Sanger sequencing revealed that this DNA was either of fungal or bacterial origin. Also, our SEM images showed the presence of endosymbionts on the tests of certain foraminiferal morphotypes (Fig. 16) indicating the presence of extra-cellular, non-target DNA. This issue posed a huge challenge for this project as also the H3A primers amplified multiple non-specific fragments. This issue has also prevailed in other recent studies (Macher et al., 2021) where three dinoflagellate symbiont clades were detected via metagenomics on the morphotype *Amphisorus* and found that these endosymbionts closely shared their genotypes with their host; furthermore, each morphotype and sampling locality had specific associated bacterial communities. It was not possible for us to solve this problem in the frame of this project, which is why we tested other PCR primers like the universal COI and HCO mitochondrial primers (Hebert et al., 2003), which were unfortunately also unsuccessful.

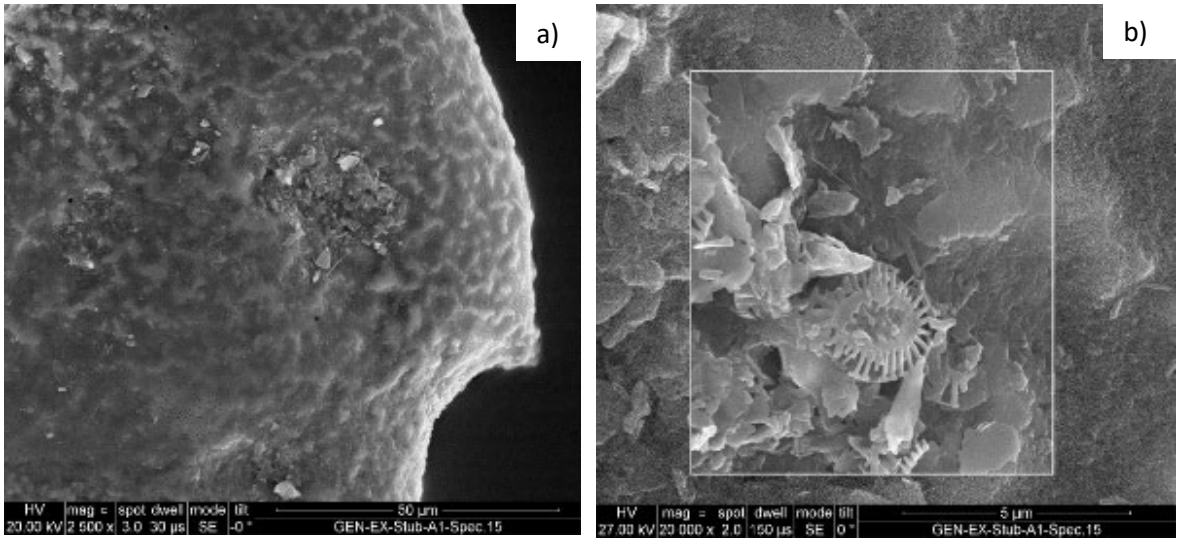


Fig. 16. SEM of Bryzalina difformis *with coccolithophores and other contamination attached to its test (see close-up on the right).*

The more specific ribosomal S14 series of primers by Pawłowska et al. (2012, 2016) were then tested using HotStartMM and DreamTaq polymerases, including various modifications to the PCR protocol (see above). We obtained six good quality DNA sequences (with a length of 580 base pairs) from the modern Dury Voe individuals which showed a strong BLAST (Altschul et al., 1990) match (e = 0) to Foraminifera DNA in online databases.

Although the S14 F3, S14 F1 and SB primers enabled the amplification of a fragment of the nuclear 18S gene ribosomal RNA of specific foraminiferal taxa from modern offshore samples, this approach was not successful for the palaeo-samples. Most likely, the latter samples contained fragmented DNA of lower quality, which would require primers which target shorter fragments or additional optimizations of the DNA extraction and PCR protocols; this was unfortunately not possible in the time and budgetary frame of GEN-EX. We can also not exclude the possibility that there were too low concentrations of foraminiferal DNA in these samples or that the DNA was too fragmentary (as suggested by Pawłowski et al., 2022 for eDNA from sediment) to enable successful PCR amplification and DNA sequencing with either classic Sanger or the modern high-throughput sequencing methods.

Additional nuclear PCR primers were also tested next to amplify other genes (i.e., alphatubulin, betatubulin, C5/138), which were unsuccessful for all samples including the modern individuals. We hypothesise that this is because the individuals were already dead at the time of collection, as demonstrated by the Spiekeroog pilot test.

Subsequent molecular tests focused on amplifying another (shorter) hyper-variable region (V9) of the foraminiferal 18S gene ribosomal RNA. Our protocol was based on a study by Amaral-Zettler et al. (2009). However, as this gene is also commonly found in bacteria, species-specific Foraminifera amplification was challenging. The PCR primers 1380/1389 and 1510 targeting V9 region were supposedly protist-specific. We applied the V9 primers to our positive control (*H. germanica*) from Spiekeroog first, where they amplified the V9 region from diatom DNA (*Navicula arenaria*) as was revealed by a BLAST search of the obtained DNA sequences, and not of the Foraminifera. It was obvious

that also these primers were unsuitable for our project. Another small-scale experiment was performed to test the V9 primers on Foraminifera from modern sediment samples from both Spiekeroog and Dury Voe using another DNA polymerase (Phusion MM). DNA was amplified, but the observed amplicons were of large and varied sizes (above 200 base pairs), indicating another unspecific amplification, possibly from bacteria (as is common in ancient DNA studies; Armbrecht et al., 2020) as the band sizes were similar to those seen with the LSU primers. Another high-fidelity DNA polymerase (Phusion Hi-Fi) together with the addition of DMSO were tried as a final test of the V9 primers on 48 samples. This test included modern, palaeo- and intertidal samples from Shetland, using *H. germanica* as a positive control. PCR amplification worked for very few samples, and the displayed band size indicated that the DNA fragment was too large to be of Foraminifera origin. We decided to not further test the V9 primers.

Table 1. In addition to the different DNA extraction methods tested, this table provides a summary of all modifications made to the PCR protocol and combinations tested between year 1 and 3 of the project.

Crushing of individual tests with micro-pestle
Changing concentration and amount of lysis buffer
Changing the temperature for overnight lysis
Use of buffer AE vs. double-distilled water
Change to DreamTaq polymerase (from Hot Start MM)
Use of universal 18S LSU PCR primers
Use of Folmer HCO/LCO COI PCR primers
Use of alternative Deoxynucleoside triphosphates (DNTPs)
Use of α-tubulin PCR primers
Use of β-tubulin PCR primers
Use of Polyubiquitin PCR primers
Use of C5/138 PCR primers
Use of 082F/2514R PCR primers
Use of 18S rRNA S14F1 and S14F3 PCR primers (successful)
Use of 18S rRNA S14F1 and SB PCR primers (successful)
Use of 18S rRNA S14F1 and S17 PCR primers
Use of 1380/1389 and 1510 V9 PCR primers
Staining of foraminiferan collected live with Rose Bengal (successful)
Use of 18S rRNA S14F1 and S15 PCR primers (successful)
Addition of Phusion Hi-Fidelity polymerase (successful)
Addition Dimethyl sulfoxide (DMSO) (successful)
Addition of Bovine serum albumin (BSA) (successful)

A final modification was made to the molecular protocol to add BSA to increase PCR yields. Using the short primers S14F1 and S15 (which are also more Foraminifera-specific than the V9 primers used by Amaral-Zettler et al., 2009), we were then able to successfully amplify the positive foraminiferal control *H. germanica* at the targeted fragment length of 190 base pairs. All previously tested primers for amplifying longer PCR products were also tested again with the addition of BSA, but results were unsuccessful. At this stage of the study, it had become clear that the shorter fragment should be targeted. Identification of the DNA sequences derived from the large PCR fragments of the palaeo-

samples from Dury Voe (DV08) and Maggie Kettle's Loch (MK1) by BLAST searches (Altschul et al., 1990) revealed that the obtained amplicons were actually derived from fish and shrimp. This confirmed that the tsunami deposit contains marine DNA but is dominated by the DNA of larger marine taxa than Foraminifera (i.e., fish and shrimp). Even high-throughput sequencing would have difficulties to provide sufficient sequencing reads of underrepresented taxa like the Foraminifera here, unless sequencing depths would be significantly increased, thereby also increasing the costs of this technique substantially.

It is noteworthy to mention that the palaeo-samples also showed a feint, shorter band of the same size as our positive control (*Cibicides lobatulus*), indicating that foraminiferal DNA may be present within these palaeo-samples, albeit in minute amounts. Alternatively, sediment chemistry could have interfered with the PCR efficiency through inhibition of the Taq polymerase (Fortin et al., 2004). The optimised. The optimised molecular protocol was not yet working at all for the intertidal sediment samples, probably because they contained very little to no foraminifera DNA, as the sampling site was heavily influenced by coarse terrestrial deposits from a debouching stream.

As a final modification, the PCR success was tested by adding BSA together with the HotStartMM polymerase to six samples including the positive control. The amplification was successful indicating that the three crucial components of the optimised PCR amplification protocol are (i) the addition of DMSO, (ii) BSA and (iii) using the shorter S15 PCR primer, while the DNA polymerase can be either the high-fidelity Phusion polymerase or the HotStartMM. These are important results for future research, also if it is intended to use high-throughput sequencing.

Fig. 17. shows that five samples produced a PCR amplicon of similar size as the positive control *C. lobatulus* (sequenced and matched 99% to Foraminifera DNA in GenBank). These five samples included: modern intertidal sediment from Dury Voe, offshore modern morphotypes 03 and 18 from Dury Voe and also one sand sample from the 1737 Chaihuín deposit.

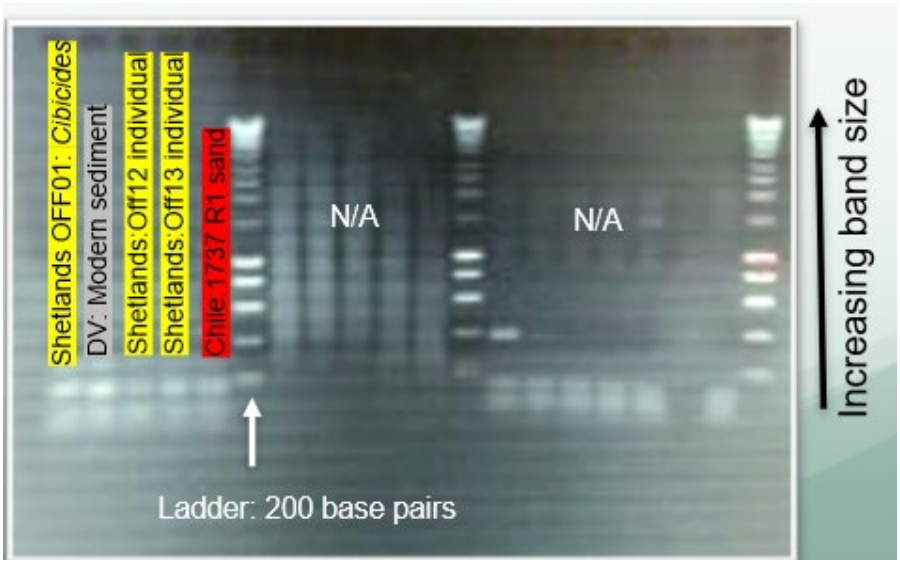


Fig. 17. Polymerase chain reaction (PCR) results on an agarose gel showing DNA amplification at 200 base pairs of a modern offshore individual foraminiferan from Shetlands, a modern-sediment sample and a palaeo-sediment sample from Chile.

Successful sequences were obtained for the modern offshore individual (OFF13, Dury Voe), which showed a 97% match to the foraminiferal taxon *Saccaminidae* sp. (Fig. 18).

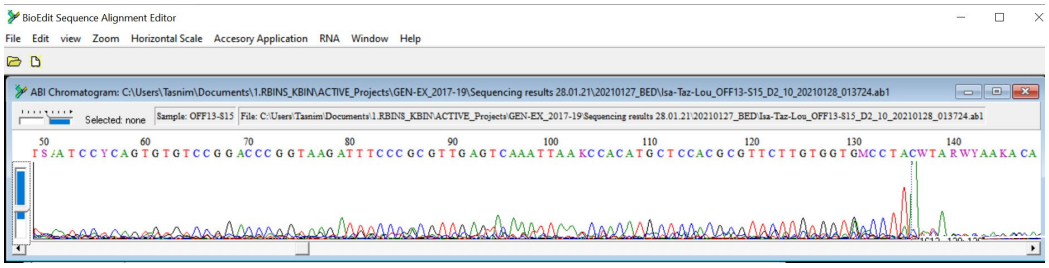


Fig 18. Chromatogram showing a DNA sequence amplified from a modern offshore individual foraminiferan from Dury Voe, Shetland.

Our most noteworthy result was the successful amplification of DNA from a 285 year-old tsunami sand layer from Chaihuín, Chile, which showed a 92.66% match to the Foraminifera *H. germanica* (Fig. 19), indicating foraminiferal DNA was present in this sediment. Given the feint PCR band observed, high-throughput sequencing of this amplicon would have not been successful with a classic setup. Post-sequencing, the exclusion of sequencing reads occurring in low numbers is a usual feature of bioinformatic pipelines analysing eDNA data (like dada2 or QIIME). These stringent filtration steps would have automatically filtered out the minute numbers of DNA sequencing reads of rare Foraminifera reads being in this amplicon and would have required the adaptation of existing bioinformatic analyses steps. This could have been countered by increasing sequencing depth and coverage, making this step more time and cost intensive. However, the required depth was well beyond the scope of the GEN-EX budget but may still be possible in future studies. Another issue for the successful application of high throughput sequencing was the lack of DNA sequences for expected/targeted benthic foraminifera species available in a reference database. Other studies have reported similar issues of eDNA approaches for understudied areas (see for example Garcia-Vazquez et al., 2021)

It is thus reasonable to conclude that foraminiferal DNA was either absent from or too degraded within the 1500-year-old tsunami deposit of the Dury Voe event. Subsequent testing and optimisation after March 2020 were not possible as the genetic laboratories at RBINS were closed on and off for a period of 18-months due to Covid regulations.

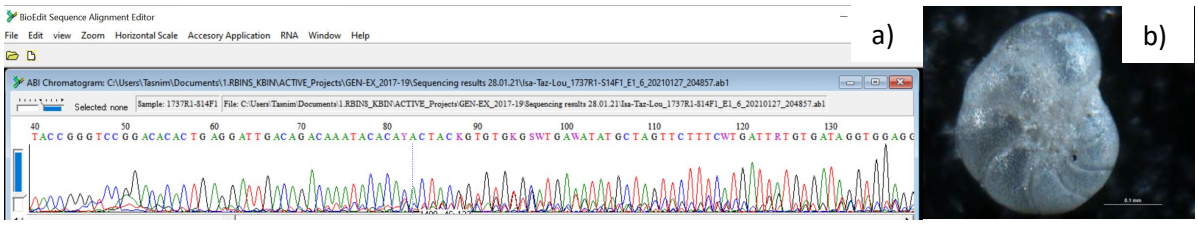


Fig 19. a) DNA chromatogram of a foraminifera sequence amplified from a 285-year-old tsunami sand layer from Chaihuín, Chile, which showed a 92.66% match to the Foraminifera species *Haynesina germanica* and b) Light microscope image of *H. germanica*.

Species identification of Foraminifera is often complex, as both morphological and molecular approaches can be challenging due to a lack of unique characters and available reference sequences (Macher et al., 2021; Perry et al., 2022). Owing to the paucity of foraminiferal DNA sequence data in

the online databases BOLD (https://boldsystems.org/), GenBank (https://www.ncbi.nlm.nih.gov/genbank), Pfr2 (http://www.oceanomics.eu/fr/publications/pfr2-a-curated-database-planktonic-foraminifera-18s-ribosomal-dna-a-resource-studies, a database dedicated to planktonic Foraminifera and not conducive to the objectives of GEN-EX) and UNIGE (http://forambarcoding.unige.ch/) plus a further underrepresentation of specifically benthic foraminiferal DNA sequences in these databases (Table 2), developing specific foraminiferal PCR primers posed a huge challenge, in which we did not yet succeed.

Table 2: A summary of the known records of foraminifera in four genetic databases.

Database	Number of foraminifera sequences/BINs/MOTUs (03/23)	Species	Benthic/Planktonic
BOLD	9 (out of 8.7 million records)	*Virgulinella fragilis* (2) Unnamed (7)	Benthic (but with endosymbionts)
GenBank	307, 176 (nucleotide, exact organism filters applied)	Foraminifera environmental sample (264423) = Most are transcribed amino acid sequences from environmental samples *Reticulomyxa filosa* (23732) *Elphidium margaritaceum* (2756) *Globigerinoides ruber* (953) *Amphistegina lobifera* (827) *Globigerinella siphonifera* (497) *Neogloboquadrina pachyderma* (442) *Globigerina bulloides* (377) *Pulleniatina obliquiloculata* (303) *Globorotalia inflata* (292) *Ammonia* sp. *genotype T6* (290) *Globigerinoides sacculifer* (286) *Globorotalia truncatulinoides* (268) *Globigerinoides elongatus* (255) *Elphidium aculeatum* (236) *Ammonia* sp. (217) *Globigerinita uvula* (208) *Globigerinita glutinata* (201) *Amphistegina lessonii* (195) *Elphidium williamsoni* (194) Unspecified taxa (10224)	Not specified
Pfr2	3322	*Beella digitata* (35) *Candeina nitida* (18) *Galietelia vivans* (5) *Globigerina bulloides* (262) *Globigerina falconensis* (8) *Globigerinella calida* (74) *Globigerinella siphonifera* (400)	Planktonic

		Globigerinita glutinata (97) *Globigerinita uvula* (190) *Globigerinoides conglobatus* (25) *Globigerinoides elongatus* (113) *Globigerinoides ruber* (210) *Globigerinoides sacculifer* (181) *Globoquadrina conglomerate* (33) *Globorotalia hirsute* (79) *Globorotalia inflata* (153) *Globorotalia menardii* (31) *Globorotalia scitula* (53) *Globorotalia* sp. (2) *Globorotalia truncatulinoides* (95) *Globorotalia tumida* (18) *Globorotalia ungulate* (39) *Globoturborotalita rubescens* (9) *Hastigerina pelagica* (166) *Hastigerinella digitata* (14) *Neogloboquadrina dutertrei* (77) *Neogloboquadrina incompta* (110) *Neogloboquadrina pachyderma* (275) *Neogloboquadrina* sp. (5) *Orbulina universa* (168) *Pulleniatina obliquiloculata* (271) *Rotaliida* sp.1 (2) *Rotaliida* sp.2 (4) *Rotaliida* sp.4 (1) *Rotaliida* sp.5 (1) *Sphaeroidinella dehiscens* (6) *Streptochilus globigerus* (19) *Turborotalita quinqueloba* (73)	
UNIGE		Offline at the time of writing	

Obtaining additional DNA sequence data is required to be able to generate a modern Foraminifera database, which will be suitable for research on extreme-wave events. Without such a database, high-throughput sequencing approaches cannot be applied.

If reference data are absent, Operational Taxonomic Units (OTUs) or Amplicon Sequencing Variants (ASVs) can be used instead (Callahan et al., 2017; Keck et al., 2018) to compare biodiversity of samples without their taxonomic identification. Unfortunately, such a strategy could not be applied to our project because our aim was not to study Foraminifera biodiversity as such, as in other studies (Pawłowska et al., 2020; Lejzerowicz et al., 2021), or to detect novel yet undescribed species, but to identify specific Foraminifera species, which are indicative for extreme-wave events. For this purpose, the construction of a reference database remains mandatory.

Nonetheless, the sequences already generated during the GEN-EX project form an important contribution to the field of tsunami research and will also alleviate the challenges of foraminiferal species identification based on the incongruences of molecular and morphological evidence. We plan to submit these to GenBank in the near future.

It is recommended that future projects ensure that living individuals are stained with Rose Bengal at the time of collection, to ensure that the DNA is not degraded and where possible, focus on a field locality where the deposit is not bracketed by acidic peat, causing dissolution of tests and rapid breakdown of the remaining (fragile) extracellular DNA. Environments which have thus far been shown to be conducive to the preservation of foraminiferal DNA are colder and oxygen poor environments such as the Arctic and the abyssal deep sea.

4. CONCLUSIONS

The molecular work conducted in the scope of GEN-EX has shed light on the metagenomic approach to tsunami research via extensive optimizations of the laboratory protocols. Although we were able to obtain sequences from individual Foraminifera and amplify foraminiferal DNA from a 1737 tsunami deposit, possible prevailing reasons for the experienced challenges in amplifying foraminiferal DNA in the palaeotsunami samples may be the presence of episymbionts, dominant DNA of non-target marine organisms and time-associated DNA degradation especially in an acidic peat environment. It is also possible that Foraminifera DNA was altogether absent from the sediment collected. The non-specificity of PCR primers is another prevailing challenge in molecular foraminiferal research (Chronopoulou et al., 2019), together with the paucity of reference DNA sequence data, which should be prioritised in future studies.

Applying classical micropalaeontological techniques, we were able to characterise the foraminiferal signature of the potential source of onshore tsunami deposits on Shetland, specifically at the locality of Dury Voe (Figs. 6,7). Further sedimentological work in the framework of GEN-EX provided evidence for the variability of storm patterns in the NE Atlantic region (Hess et al., 2023) and substantiated the evidence for a late Holocene tsunami impact on Shetland (Engel et al., 2023).

5. DISSEMINATION AND VALORISATION

Publications

A1 publications (in review)

- Hess, K., Engel, M., Patel, T., Vakhrameeva, P., Koutsodendris, A., Klemt, E., Hansteen, T.H., Kempf, P., Dawson, S., Schön, I., Heyvaert, V.M.A., in review. A 1500-years record of North Atlantic storminess from the Shetland Islands (UK). Journal of Quaternary Science (JQS-22-0143) --> Preprint available at Research Square: https://doi.org/10.21203/rs.3.rs-2731397/v1
- Engel, M., Hess, K., Dawson S., Patel, T., Koutsodendris, A., Vakhrameeva, P., Klemt, E., Kempf, P., Schön I., Heyvaert V.M.A., in review. The late Holocene tsunami in the Shetland Islands (UK) identified in Loch Flugarth, north Mainland. Boreas (BOR-008-2023) --> Preprint available at Research Square: https://doi.org/10.21203/rs.3.rs-2750922/v1

Published datasets

- Hess, K., Engel, M., Koutsodendris, A., 2023. Sedimentological storm and tsunami record of Loch Flugarth, Shetland Islands (UK). heiDATA. https://doi.org/10.11588/data/QJEZHT

Peer-reviewed book chapters

- Engel, M., Schön I., Patel, T., Pawłowski, J., Szczuciński, W., Dawson, S., Garrett, E., Heyvaert, V.M.A., 2020. Paleogenetic approach in tsunami deposit studies. In: Engel, M., Pilarczyk, J., May, S.M., Brill, D., Garrett, E. (eds.), Geological records of tsunamis and other extreme waves. Elsevier, Amsterdam, pp. 427-442. https://doi.org/10.1016/B978-0-12-815686-5.00020-1 (--> edited book nominated for the 2021 Prose Award "Physical Sciences and Mathematics – Earth Science")

Abstracts

1. Engel, M., Hess, K., Patel, T., Kempf, P., Koutsodendris, A., Vakhrameeva, P., Klemt, E., Dawson, S., Schön, I., Heyvaert, V.M.A.: New sedimentary evidence for the ~1500 BP tsunami on the Shetland Islands. INQUA Congress, Rome, 13th–20th July 2023. Abstract #2829 (poster).

2. Hess, K., Engel, M., Patel, T., Vakhrameeva, P., Koutsodendris, A., Klemt, E., Hansteen, T.H., Kempf, P., Dawson, S., Schön, I., Heyvaert V.M.A.: Varying seasonal patterns of NE Atlantic storminess inferred from a 1500-years sediment record on the Shetland Islands (UK). INQUA Congress, Rome, 13th–20th July 2023. Abstract #2139 (poster).

3. Engel, M., Hess, K., Patel, T., Vakhrameeva, P., Koutsodendris, A., Klemt, E., Hansteen, T.H., Kempf, P., Dawson, S., Schön, I., Heyvaert, V.M.A.: A late Holocene record of tsunami and North Atlantic storminess from the Shetland Islands (UK). BELQUA Annual Scientific Workshop, Brussels, 6–7th March 2023 (oral).

4. Hess, K., Engel, M., Patel, T., Dawson, S., Oetjen, J., Koutsodendris, A., Vakhrameeva, P., Schön I., Heyvaert V.M.A.: Historical storm frequency on the Shetland Islands (UK) – Insights from lake sediment

cores and coastal wave modelling. Mid-European Geomorphology Meeting 2021, Munich, 6th–9th November 2021 (poster).

5. Hess, K., Engel, M., Oetjen, J., Patel, T., Koutsodendris, A., Vakhrameeva, P., Schön I., Dawson, S., Heyvaert V.M.A.: A 1500 years-record of North Atlantic storminess from the Shetland Islands (UK) – preliminary insights. 7th International Geologica Belgica meeting, Tervuren, Belgium, 14th–17th September 2021 (oral).

6. Engel, M., Patel, T., Pint, A., Dawson, S., Schön, I., Heyvaert, V.M.A.: Metagenomics of tsunami deposits: developments and challenges from a case study on the Shetland Islands (UK). 7th International Geologica Belgica meeting, Tervuren, Belgium, 14th–17th September 2021 (poster).

7. Hess, K., Engel, M., Oetjen, J., Patel, T., Koutsodendris, A., Vakhrameeva, P., Schön, I., Dawson, S., Heyvaert V.M.A.: Historical storm frequency on the Shetland Islands (UK) – Preliminary insights from lake sediment cores and coastal wave modelling. 38th Annual Meeting of the German Working Group on Geography of Oceans and Coasts, Hannover, 01st–02nd June 2021 (poster, online).

8. Hess, K., Engel, M., Oetjen, J., Patel, T., Schön I., Dawson, S., Heyvaert V.M.A.: Historical records of storm frequency on the Shetland Islands (UK) – Preliminary insights from lake sediment cores and coastal wave modelling. EGU General Assembly, Vienna, 08th–13th April 2021, Abstract EGU21-8773, https://doi.org/10.5194/egusphere-egu21-8773 (PICO, online).

9. Engel, M., Patel, T., Dawson, S., Pint, A., Schön, I., Heyvaert, V.M.A.: Metagenomics of tsunami deposits: developments, challenges and recommendations from a case study on the Shetland Islands (UK). EGU General Assembly, Vienna, 4th–8th May 2020, Abstract EGU2020-18238, https://doi.org/10.5194/egusphere-egu2020-18238 (PICO, online).

10. Patel, T., Engel, M., Dawson, S., Pint, A., Schön, I., Heyvaert, V.M.A.: Metagenomics of tsunami deposits: developments, challenges and recommendations from a case study on the Shetland Islands (UK). BELQUA workshop, Brussels, 26th March 2020 (oral communication accepted, conference cancelled due to COVID19).

11. Heyvaert, V.M.A., Engel, M., Patel, T., Pint, A., Kempf, P., Dawson, S., Schön, I.: GEN-EX – Application of metagenomic analyses to extreme wave deposits in the Shetland Islands, UK., International conference "From the North Sea Lowlands to the Celtic Shelf Edge", Utrecht, 18th–20th November 2019 (poster).

12. Engel, M., Patel, T., Dawson, S., Pint, A., Kempf, P., Schön, I., Heyvaert, V.M.A.: Metagenomics of Storegga tsunami deposits on Shetland, UK. International Workshop "Drowned paleo-landscapes. Current archaeological and natural scientific research in the Wadden Sea and the North Sea basin", Delmenhorst, Germany, 19th–20th September 2019 (poster).

13. Patel, T., Engel, M., Dawson, S., Pint, A., Garrett, E., Szczuciński, W., Kempf, P., Schön, I., Heyvaert, V.M.A.: Metagenomics of tsunami deposits using eDNA: First results from the Shetland Islands, U.K. 20th INQUA Congress, Dublin, 25th–31st July 2019, Abstract O-3162 (oral).

14. Engel, M., Patel, T., Dawson, S., Pint, A., Kempf, P., Costa, P.J.M., Schön, I., Heyvaert, V.M.A.: Selective representation of sediment sources in tsunami deposits from the Shetland Islands (U.K.). 20th INQUA Congress, Dublin, 25th–31st July 2019, Abstract P-3045 (poster).

15. Engel, M., Patel, T., Dawson, S., Pint, A., Kempf, P., Costa, P.J.M, Schön, I., Heyvaert, V.M.A., 2019. Selective representation of sediment sources in tsunami deposits from the Shetland Islands (U.K.). 37th Annual Meeting of the German Working Group on Geography of Oceans and Coasts, Cologne, 09th–11th May 2019 (poster).

16. Engel, M., Patel, T., Dawson, S., Pint, A., Garrett, E.G., Szczuciński, W., Kempf, P., Schön, I., Heyvaert, V.M.A.: Metagenomics of tsunami deposits. Central European Conference on Geomorphology and Quaternary Sciences (joint conference of German Working Group of Geomorphology and DEUQUA), Giessen, Germany, 23th–27th September 2018 (poster).

17. Engel, M., Schön, I., Patel, T., Dawson, S., Garrett, E., Szczuciński, W., Kempf, P., Heyvaert, V.M.A.: Tracing ancient DNA of foraminifera in tsunami deposits (GEN-EX). 6th Geologica Belgica Meeting, Leuven, 12th–14th September 2018 (poster).

18. Engel, M., Schön, I., Patel, T., Dawson, S., Garrett, E., Heyvaert, V.M.A.: Gen-EX – Metagenomics of extreme-wave events. Multidisciplinary Workshop on Disaster Resilience in the 21st Century, Brussels, 11 December 2017 (poster).

Summer school

The summer school was planned but not executed owing to COVID.

Supervision of students and output

Master of Science thesis (Heidelberg University) – Katharina Hess: Historical records of North Atlantic storminess on the Shetland Islands (UK) Sedimentological and geochemical analyses of overwash processes from a coastal lake (completed in Dec 2021)

Bachelor of Science thesis (Heidelberg University) – Nelina Nussberger: Heavy mineral analysis of tsunami deposits at Dury Voe (Shetland Islands) and their potential sediment source (working title, ongoing).

Other outreach activities

26.10.2018: Funding application for a GEN-EX project valorisation as an EDGE conference, €26,000, not selected for funding.

6. PERSPECTIVES

Characterisation of Foraminifera communities in palaeo-samples has proven challenging as intact fossils are rare. Especially in peat-rich environments such as the Shetland Islands, DNA degrades with

time owing to microbial enzymatic activity, mechanical shearing and spontaneous chemical reactions such as hydrolysis and oxidation (Lindahl, 1993).

It has been recently demonstrated that molecular techniques can aid the differentiation of storm overwash and tsunami flooding using microbial communities (Yap et al., 2021, 2023), using 16S and 18S rRNA metabarcoding. Therefore, with the recommendations presented here, the protocol may still be adapted for a similar differentiation using Foraminifera. First promising results exist from Japan (Szczuciński et al., 2016). Indeed, it has been demonstrated in this study that targeting a length of 200 base pairs in the context of palaeo-tsunami deposits, may be too long. Thus, it is recommended that eDNA of a maximum of 50 base pairs is targeted following the protocol of Kjær et al. (2021). It is furthermore recommended that future studies apply a "shotgun sequencing" approach to obtain the eDNA signal in its entirety (i.e., including endosymbionts and bacterial communities), which has only thus far been successfully demonstrated by a sole study (Macher et al., 2021). Table 1 shows the culmination of knowledge gained regarding the application of molecular methods to foraminiferal DNA in sediments.

Most offshore samples from the Shetland Islands were rich in skeletal carbonate and in the deeper samples foraminiferal concentration was quite high, whereas intertidal and very shallow subtidal to lagoonal samples (Maggie Kettle's Loch and Flugarth Loch) showed low concentrations or even entire absence. This could indicate that Foraminifera were absent in these samples or that the samples had a low pH (from the bracketing peat layers), which could have led to the degradation of any eDNA. Furthermore, for example Pawłowski et al. (2022) described that sediment chemistry can affect the efficiency of DNA extractions and PCR amplification – even if extracellular DNA was present in our samples, it is possible that the chemical characteristics of the acidic bracketing sediment made the application of molecular methods very difficult. This is reflected by most DNA in marine benthic ecosystem studies being represented by extracellular DNA (see Pawłowski et al., 2022). The few new studies where Foraminifera have been successfully investigated from sediments with molecular methods come from very cold environments like the Arctic (Pawłowska et al., 2020) and the deep sea (Lejzerowicz et al., 2021) with limited DNA degradation because of the environmental conditions there. It is noteworthy that all of the aforementioned studies adding to the knowledge base obtained throughout the scope of the GEN-EX project, came long after the GEN-EX project was designed. Surprisingly, no standard has been published until 2022 on the application of eDNA for soft marine sediments, and even this recommendation mainly focuses on the first steps of the process, field sampling, fixation of samples and DNA extraction from sediments (Pawłowski et al., 2022), and not the challenging downstream bioinformatic optimisations.

In sum, the project has revealed that extraction methods and PCR amplification protocols must be modified individually for each of the different sample types, i.e., modern offshore, modern intertidal and palaeotsunami samples, an important consideration for this type of metagenomic research in future.

8. ACKNOWLEDGEMENTS

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9. REFERENCES

Aedo, D., Melnick, D., Garrett, E., Pino, M. 2021. Origen y distribución de depósitos de tsunami en la marisma de Chaihuín (40°S/73,5°W), Chile. Andean Geology, 48, 125–152. https://doi.org/10.5027/andgeoV48n1-3258

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. 1990. Basic local alignment search tool. Journal of Molecular Biology, 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amaral-Zettler L.A., McCliment E.A., Ducklow H.W., Huse S.M. 2009. A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes. PLOS ONE, 4, e6372. https://doi.org/10.1371/journal.pone.0006372

Armbrecht, L.H., Coolen, M.J.L., Lejzerowicz, F., George, S.C., Negandhi, K., Suzuki, Y., Young, J., Foster, N.R., Armand, L.K., Cooper, A., Ostrowski, M., Focardi, A., Stat, M., Moreau, J.W., Weyrich, L.S. 2019. Ancient DNA from marine sediments: Precautions and considerations for seafloor coring, sample handling and data generation. Earth-Science Reviews, 196, 102887. https://doi.org/10.1016/j.earscirev.2019.102887

Baker, A., Hellstrom, J.C., Kelly, B.F.J., Mariethoz, G., Trouet, V. 2015. A composite annual-resolution stalagmite record of North Atlantic climate over the last three millennia. Scientific Reports, 5, 10307. https://doi.org/10.1038/srep10307

Blaauw, M., Christen, J.A. 2011. Flexible paleoclimate age-depth models using an auto-regressive gamma process. Bayesian Analysis, 6, 457–474. https://doi.org/10.1214/11-BA618

Bondevik, S., Løvholt, F., Harbitz, C., Mangerud, J., Dawson, A., Svendsen, J.I. 2005a. The Storegga Slide tsunami—comparing field observations with numerical simulations. Marine and Petroleum Geology, 22, 195–208. https://doi.org/10.1016/j.marpetgeo.2004.10.003

Bondevik, S., Mangerud, J., Dawson, S., Dawson, A., Lohne, Ø. 2005b. Evidence for three North Sea tsunamis at the Shetland Islands between 8000 and 1500 years ago. Quaternary Science Reviews, 24, 1757–1775. https://doi.org/10.1016/j.quascirev.2004.10.018

Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., Sweet, M. 2021. Improving the reliability of eDNA data interpretation. Molecular Ecology Resources, 21, 1422–1433. https://doi.org/10.1111/1755-0998.13367

Callahan, B., McMurdie, P., Holmes, S. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal, 11, 2639–2643. https://doi.org/10.1038/ismej.2017.119

Chronopoulou, P.M., Salonen, I., Bird, C., Reichart, G.J., Koho, K.A. 2019. Metabarcoding insights into the trophic behavior and identity of intertidal benthic Foraminifera. Frontiers in Microbiology, 10, 1169. https://doi.org/10.3389/fmicb.2019.01169

Cordier, T., Alonso-Sáez, L., Apothéloz-Perret-Gentil, L., Aylagas, E., Bohan, D.A., Bouchez, A., Chariton, A., Creer, S., Frühe, L., Keck, F., Keeley, N., Laroche, O., Leese, F., Pochon, X., Stoeck, T., Pawłowski, J., Lanzén, A. 2021. Ecosystems monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap. Molecular Ecology, 30, 2937–2958. https://doi.org/10.1111/mec.15472

Dawson, A.G., Dawson, S., Bondevik, S. 2006. A late Holocene tsunami at Basta Voe, Yell, Shetland Islands. Scottish Geographical Journal, 122, 100–108. https://doi.org/10.1080/00369220600917404

Dry, F.T., Robertson, J.S. 1982. Soil Survey of Scotland - Orkney and Shetland. Aberdeen.

Engel, M., Oetjen, J., May, S.M., Brückner, H. 2016. Tsunami deposits of the Caribbean – Towards an improved coastal hazard assessment. Earth-Science Reviews, 163, 260–296. https://doi.org/10.1016/j.earscirev.2016.10.010

Engel, M., Hess, K., Dawson, S., Patel, T., Koutsodendris, A., Vakhrameeva, P., Klemt, E., Kempf, P., Schön, I., Heyvaert, V.M.A. 2023. The late Holocene tsunami in the Shetland Islands (UK) identified in Loch Flugarth, north Mainland. Preprint available at Research Square. https://doi.org/10.21203/rs.3.rs-2750922/v1

Fortin, N., Beaumier, D., Lee, K., Greer, C.W. 2004. Soil washing improves the recovery of total community DNA from polluted and high organic content sediments. Journal of Microbiological Methods, 56, 181–91. https://doi.org/10.1016/j.mimet.2003.10.006

Garcia-Vazquez, E., Georges, O., Fernandez, S., Ardura, A. 2021. eDNA metabarcoding of small plankton samples to detect fish larvae and their preys from Atlantic and Pacific waters. Scientific Reports, 11, 7224. https://doi.org/10.1038/s41598-021-86731-z

Giraudeau, J., Grelaud, M., Solignac, S., Andrews, J.T., Moros, M., Jansen, E. 2010. Millennial-scale variability in Atlantic water advection to the Nordic Seas derived from Holocene coccolith concentration records. Quaternary Science Reviews, 29, 1276–1287. https://doi.org/10.1016/j.quascirev.2010.02.014

Goff, J., Chagué-Goff, C., Nichol, S., Jaffe, B., Dominey-Howes, D. 2012. Progress in palaeotsunami research. Sedimentary Geology, 243–244, 70–88. https://doi.org/10.1016/j.sedgeo.2011.11.002

Gold, Z., Wall, A.R., Schweizer, T.M., Pentcheff, N.D., Curd, E.E., Barber, P.H., Meyer, R.S., Wayne, R., Stolzenbach, K., Prickett, K., Luedy, J., Wetzer, R. 2022. A manager's guide to using eDNA metabarcoding in marine ecosystems. PeerJ, 10, e14071. https://doi.org/10.7717/peerj.14071

Goslin, J., Clemmensen, L.B. 2017. Proxy records of Holocene storm events in coastal barrier systems: Storm-wave induced markers. Quaternary Science Reviews, 174, 80–119. https://doi.org/10.1016/j.quascirev.2017.08.026

Hansom, J.D., Hall, A.M. 2009. Magnitude and frequency of extra-tropical North Atlantic cyclones: A chronology from cliff-top storm deposits. Quaternary International, 195, 42–52. https://doi.org/10.1016/j.quaint.2007.11.010

Harrison, J.B., Sunday, J.M., Rogers, S.M. 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proceedings of the Royal Society B, 286, 20191409. https://doi.org/10.1098/rspb.2019.1409

Hawkes, A.D. 2020. Foraminifera in tsunami deposits. In: Engel, M., Pilarczyk, J., May, S.M., Brill, D., Garrett, E. (eds.) Geological records of tsunamis and other extreme waves. Elsevier, Amsterdam, pp. 239-259. https://doi.org/10.1016/B978-0-12-815686-5.00012-2

Heiri, O., Lotter, A.F., Lemcke, G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of Paleolimnology, 25, 101–110. https://doi.org/10.1023/A:1008119611481

Hebert, P.D.N., Cywinska, A., Ball, S. L., deWaard, J.R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B, 270, 313–321. https://doi.org/10.1098/rspb.2002.2218

Hess, K., Engel, M., Patel, T., Vakhrameeva, P., Koutsodendris, A., Klemt, E., Hansteen, T.H., Kempf, P., Dawson, S., Schön, I., Heyvaert, V.M.A. 2023. A 1500-years record of North Atlantic storminess from the Shetland Islands (UK). Preprint available at Research Square. https://doi.org/10.21203/rs.3.rs-2731397/v1

Hocking, E.P., Garrett, E., Aedo, D. Carvajal, M., Melnick, D. 2021. Geological evidence of an unreported historical Chilean tsunami reveals more frequent inundation. Communications Earth & Environment, 2, 245. https://doi.org/10.1038/s43247-021-00319-z

Kjær, K.H., Pedersen, M.W., De Sanctis, B., De Cahsan, B., Korneliussen, T.S., Michelsen, C.S., Sand, K.K., Jelavić, S., Ruter, A.H., Schmidt, A.M.A., Kjeldsen, K.K., Tesakov, A.S., Snowball, I., Gosse, J.C., Alsos, I.G., Wang, Y., Dockter, C., Rasmussen, M., Jørgensen, M.E., Skadhauge, B., Prohaska, A., Kristensen, J.Å., Bjerager, M., Allentoft, M.E., Coissac, E., PhyloNorway Consortium, Rouillard, A., Simakova, A., Fernandez-Guerra, A., Bowler, C., Macias-Fauria, M., Vinner, L., Welch, J.J., Hidy, A.J., Sikora, M., Collins, M.J., Durbin, R., Larsen, N.K., Willerslev, E. 2022. A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. Nature, 612, 283–291. https://doi.org/10.1038/s41586-022-05453-y

Keck, F., Vasselon, V., Rimet, F., Bouchez, A., Kahlert, M. 2018. Boosting DNA metabarcoding for biomonitoring with phylogenetic estimation of operational taxonomic units' ecological profiles. Molecular Ecology Resources, 18, 1299–1309. https://doi.org/10.1111/1755-0998.12919

Lamb, H.H. 1991. Historic storms of the North Sea, British Isles and Northwest Europe. Cambridge University Press, Cambridge.

Lejzerowicz, F., Gooday, A.J., Barrenechea Angeles, I., Cordier, T., Morard, R., Apothéloz-Perret-Gentil, L., Lins, L., Menot, L., Brandt, A., Levin, L.A., Martinez Arbizu, P., Smith, C.R., Pawłowski, J. 2021. Eukaryotic biodiversity and spatial patterns in the Clarion-Clipperton Zone and other abyssal regions: insights from sediment DNA and RNA metabarcoding. Frontiers in Marine Science, 8, 671033. https://doi.org/10.3389/fmars.2021.671033

Lindahl, T. 1993. Instability and decay of the primary structure of DNA. Nature, 362, 709–715. https://doi.org/10.1038/362709a0

Long, D. 2015. A Catalogue of tsunamis reported in the UK. British Geological Survey Internal Report IR/15/043. URL: http://nora.nerc.ac.uk/id/eprint/513298 (last access: 03 Dec 2022).

Mac Conamhna, O.A.P. 2023. A seismic tsunami in the Irish annals, recorded at Iona in October 720. Marine Geology, 456, 106973. https://doi.org/10.1016/j.margeo.2022.106973

Macher, J.-N., Prazeres, M., Taudien, S., Jompa, J., Sadekov, A., Renema, W. 2021. Integrating morphology and metagenomics to understand taxonomic variability of *Amphisorus* (Foraminifera, Miliolida) from Western Australia and Indonesia. PLOS ONE, 16, e0244616. https://doi.org/10.1371/journal.pone.0244616

Mamo, B., Strotz, L., Dominey-Howes, D. 2009. Tsunami sediments and their foraminiferal assemblages. Earth-Science Reviews, 96, 263–278. https://doi.org/10.1016/j.earscirev.2009.06.007

Meeker, L.D., Mayewski, P.A. 2002. A 1400-year high-resolution record of atmospheric circulation over the North Atlantic and Asia. The Holocene, 12, 257–266. https://doi.org/10.1191/0959683602hl542ft

Orme, L.C., Reinhardt, L., Jones, R.T., Charman, D.J., Barkwith, A., Ellis, M.A. 2016. Aeolian sediment reconstructions from the Scottish Outer Hebrides: Late Holocene storminess and the role of the North Atlantic Oscillation. Quaternary Science Reviews, 132, 15–25. https://doi.org/10.1016/j.quascirev.2015.10.045

Perry, I., Jâms, I.B., Casas-Mulet, R., Hamutoko, J., Marchbank, A., Lendelvo, S., Naomab, E., Mapani, B., Creer, S., Wanke, H., Durance, I., Kille, P. 2022. Challenges to implementing environmental-DNA monitoring in Namibia. Frontiers in Environmental Science, 9, 773991. https://doi.org/10.3389/fenvs.2021.773991

Pawłowska, J., Lejzerowicz, F., Esling, P., Szczuciński, W., Zajączkowski, M., Pawłowski, J. 2014. Ancient DNA sheds new light on the Svalbard foraminiferal fossil record of the last millennium. Geobiology, 12, 277–288. https://doi.org/10.1111/gbi.12087

Pawłowska, J., Zajączkowski, M., Łącka, M., Lejzerowicz, F., Esling, P., Pawłowski, J. 2016. Palaeoceanographic changes in Hornsund Fjord (Spitsbergen, Svalbard) over the last millennium: new insights from ancient DNA. Climate of the Past, 12, 1459–1472. https://doi.org/10.5194/cp-12-1459-2016

Pawłowska, J., Łącka, M., Kucharska, M., Pawłowski, J., Zajączkowski, M. 2020. Multiproxy evidence of the neoglacial expansion of Atlantic water to eastern Svalbard. Climate of the Past, 16, 487–501. https://doi.org/10.5194/cp-16-487-2020

Pawłowski, J., Holzmann, M. 2002. Molecular phylogeny of Foraminifera a review. European Journal of Protistology 38, 1–10. https://doi.org/10.1078/0932-4739-00857

Pawłowski, J., Bruce, K., Panksep, K., Aguirre, F.I., Amalfitano, S., Apothéloz-Perret-Gentil, L., Baussant, T., Bouchez, A., Carugati, L., Cermakova, K., Cordier, T., Corinaldesi, C., Costa, F.O., Danovaro, R., Dell'Anno, A., Duarte, S., Eisendle, U., Ferrari, B.J.D., Frontalini, F., Frühe, L., Haegerbaeumer, A., Kisand, V., Krolicka, A., Lanzén, A., Leese, F., Lejzerowicz, F., Lyautey, E., Maček, I., Sagova-Marečková, M., Pearman, J.K., Pochon, X., Stoeck, T., Vivien, R., Weigand, A., Fazi, S. 2022. Environmental DNA metabarcoding for benthic monitoring: A review of sediment sampling and DNA extraction methods. Science of the Total Environment, 818, 151783. https://doi.org/10.1016/j.scitotenv.2021.151783

Pilarczyk, J.E., Dura, T., Horton, B.P., Engelhart, S.E., Kemp, A.C., Sawai, Y. 2014. Microfossils from coastal environments as indicators of paleo-earthquakes, tsunamis and storms. Palaeogeography, Palaeoclimatology, Palaeoecology, 413, 144–157. https://doi.org/10.1016/j.palaeo.2014.06.033

Rodríguez-Ezpeleta, N., Zinger, L., Kinziger, A., Bik, H.M., Bonin, A., Coissac, E., Emerson, B.C., Lopes, C.M., Pelletier, T.A., Taberlet, P., Narum, S. 2021. Biodiversity monitoring using environmental DNA. Molecular Ecology Resources, 21, 1405–1409. https://doi.org/10.1111/1755-0998.13399

Smith, D.E. 1993. Sullom Voe. In: Birnie, J., Gordon, J., Bennett, K., Hall, A. (eds.) The Quaternary of Shetland (Field Guide). Quaternary Research Association, London, p. 56.

Seneviratne, S.I., Zhang, X., Adnan, M., Badi, W., Dereczynski, C., Di Luca, A., Ghosh, S., Iskandar, I., Kossin, J., Lewis, S., Otto, F., Pinto, I., Satoh, M., Vicente-Serrano, S.M., Wehner, M., Zhou, B. 2021. Weather and climate extreme events in a changing climate. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, O., Yu,

R., Zhou B. (eds.) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York, pp. 1513–1766. https://doi.org/10.1017/9781009157896.013

Szczuciński, W., Pawłowska, J., Lejzerowicz, F., Nishimura, Y., Kokociński, M., Majewski, W., Nakamura, Y. & Pawlowski, J., 2016. Ancient sedimentary DNA reveals past tsunami deposits. Marine Geology, 381, 29–33. https://doi.org/10.1016/j.margeo.2016.08.006

Tappin, D.R., Long, D.L., Carter, G.D.O., 2015. Shetland Islands Field Trip May 2014 – Summary of Results. British Geological Survey Open Report, OR/15/017.

Trouet, V., Esper, J., Graham, N.E., Baker, A., Scourse, J.D., Frank, D.C., 2009. Persistent positive North Atlantic oscillation mode dominated the Medieval Climate Anomaly. Science, 324, 78–80. https://doi.org/10.1126/science.1166349

Uchida, J.-I., Fujiwara, O., Hasegawa, S., Kamataki, T. 2010. Sources and depositional processes of tsunami deposits: analysis using foraminiferal tests and hydrodynamic verification. Island Arc, 19, 427–442. https://doi.org/10.1111/j.1440-1738.2010.00733.x

Weiss, R., Bourgeois, J. 2012. Understanding sediments – reducing tsunami risk. Science, 336, 1117–1118. https://doi.org/10.1126/science.1221452

Yap, W., Switzer, A.D., Gouramanis, C., Marzinelli, E., Wijaya, W., Yan, Y.T., Dominey-Howes, D., Labbate, M., Srinivasalu, S., Jankaew, K., Lauro, F.M. 2021. Environmental DNA signatures distinguish between tsunami and storm deposition in overwash sand. Communications Earth & Environment, 2, 129. https://doi.org/10.1038/s43247-021-00199-3

Yap, W., Switzer, A.D., Gouramanis, C., Horton, B.P., Marzinelli, E.M., Wijaya, W., Yan, Y.T., Dominey-Howes, D., Labbate, M., Jankaew, K., Lauro, F.M. 2023. Investigating geological records of tsunamis in Western Thailand with environmental DNA. Marine Geology, 457, 106989. https://doi.org/10.1016/j.margeo.2023.106989

Yawsangratt, S., Szczuciński, W., Chaimanee, N., Chatprasert, S., Majewski, W., Lorenc, S. 2012. Evidence of probable paleotsunami deposits on Kho Khao Island, Phang Nga Province, Thailand. Natural Hazards, 63, 151–163. https://doi.org/10.1007/s11069-011-9729-4

Zinger, L., Bonin, A., Alsos, I.G., Bálint, M., Bik, H., Boyer, F., Chariton, A.A., Creer, S., Coissac, E., Deagle, B.E., De Barba, M., Dickie, I.A., Dumbrell, A.J., Ficetola, G.F., Fierer, N., Fumagalli, L., Gilbert, M.T.P., Jarman, S., Jumpponen, A., Kauserud, H., Orlando, L., Pansu, J., Pawłowski, J., Tedersoo, L., Thomsen, P.F., Willerslev, E., Taberlet, P. 2019. DNA metabarcoding – Need for robust experimental designs to draw sound ecological conclusions. Molecular Ecology, 28, 1857–1862. https://doi.org/10.1111/mec.15060

ANNEXES

None.