Metagenomics of tsunami deposits: Developments, challenges and recommendations from a case study on the Shetland Islands (UK)

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Geological records of extreme-wave events: Extreme-wave events (tsunamis, storm surges/waves) impose significant hazards to coastal communities worldwide. Onshore deposits from these events enhance our understanding of long-term frequency-magnitude patterns, which are usually not covered by historical and instrumental documentation. Such perspectives are crucial for successful coastal hazard assessments and consequential efforts to mitigate against losses.

There are a range of **typical signatures of tsunami deposits**, which significantly overlap with the characteristics of storm deposits, making a differentiation between both processes difficult (Goff et al., 2012; Engel et al., 2016). Tsunami signatures include:

Erosional basal contacts | Basal load structures | Basal traction carpet | Buried plants or soils | Rip-up clasts | Landward fining | Cross-bedding | Marine geochemical signature in terrestrial setting | Multimodal grain-size distribution | Poorer sorting | Heavy mineral lamination | One or several fining-upward sequences with mud caps coinciding with number of tsunami waves or even representing backwash, potentially intercalated by ungraded sections | Macro-/**microfossil remains** → broad range of habitats and taphonomic states

Foraminifera as indicators of tsunami deposits: Foraminifera are the most commonly used microfossils in studies of extreme-wave deposits, as they show clear depth-related zonation. Tsunami deposits are often characterised by allochthonous marine assemblages, mostly dominated by shallow marine to intertidal taxa as well as general changes in test concentration, taphonomy, diversity, size, or adult/juvenile ratios compared to background sediments. They may include taxa from outer shelf to upper bathyal depths or planktonic open marine forms, whilst a dominance of brackish/saltmarsh taxa is indicative of tsunami backwash. Tests are often broken or abraded. The presence of taxa from below the storm-wave base may even help to distinguish between storm and tsunami deposits (Engel et al., 2016) → **However, dissolution of microfossils often prevents identification and diminishes their value as a proxy.**

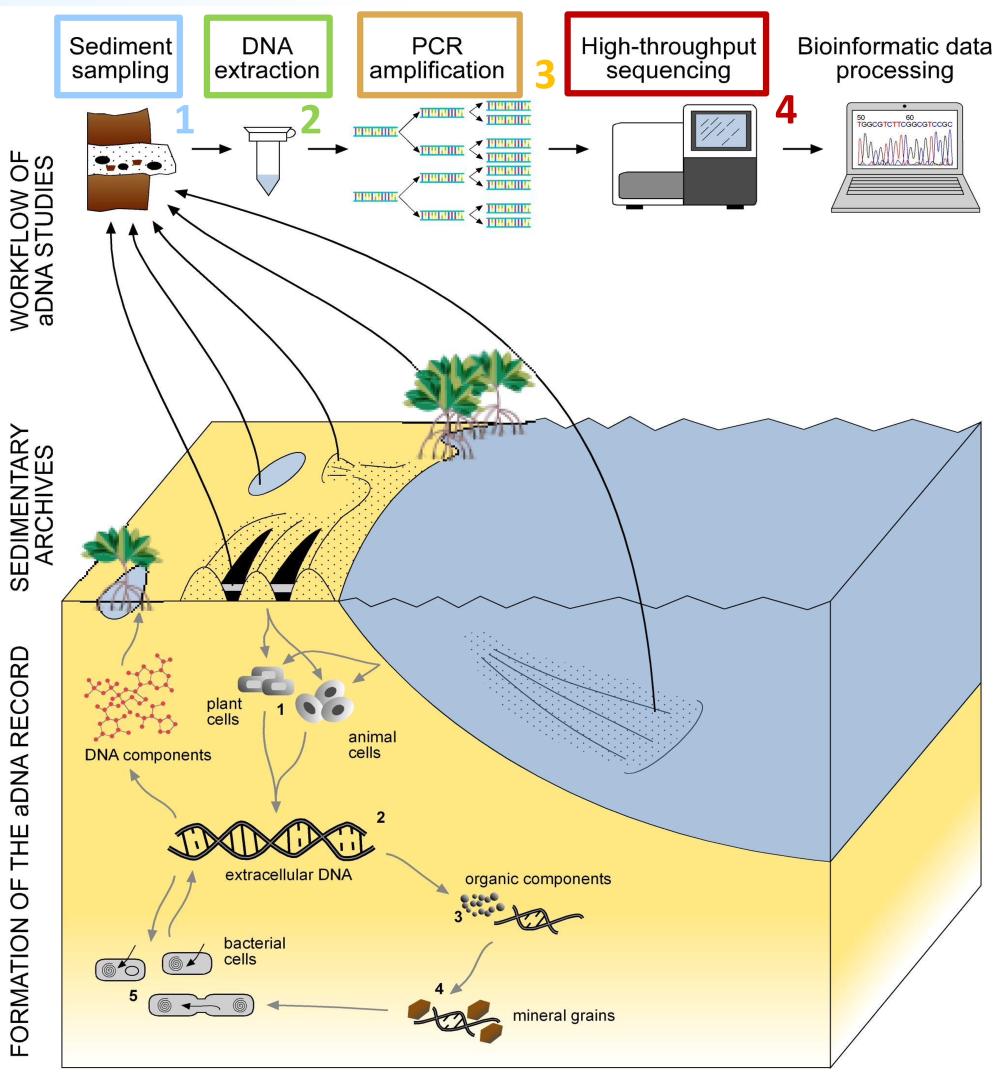


***Fig. 1**: Formation of the eDNA record after deposition in tsunami sediment archives combined with a workflow of the palaeogenomic analyses (Engel et al., 2020). The workflow of eDNA studies comprises the sampling of the tsunami deposit and subsequent extraction of DNA using extraction kits specific to the sample type. The extracted DNA is amplified with PCR and amplicons sequenced on high-throughput (mostly Illumina) platforms, before data processing (quality filtering, removal of errors, trimming, sequence sorting, analyses with bioinformatic pipelines). The final step comprises the identification of operational taxonomic units (OTUs) and further taxonomic interpretation (Thomsen & Willerslev, 2015).*

Background and aims of the GEN-EX project: Metagenomics (or environmental genomics) is sequencing DNA directly from environmental samples, where the genetic material of organisms may be preserved in sediment records covering tens of thousands of years (Fig. 1) (Thomsen & Willerslev, 2015). Foraminifera are the first group to have been identified successfully in palaeo-tsunami deposits by their environmental DNA (eDNA) (Szczuciński et al., 2016). To address the issue of test degradation, **GEN-EX will use high-throughput (Illumina), metagenomic sequencing techniques to address the issue of post-depositional test degradation by detecting and identifying Foraminifera in onshore tsunami sand layers, where tests have been lost through chemical weathering.**

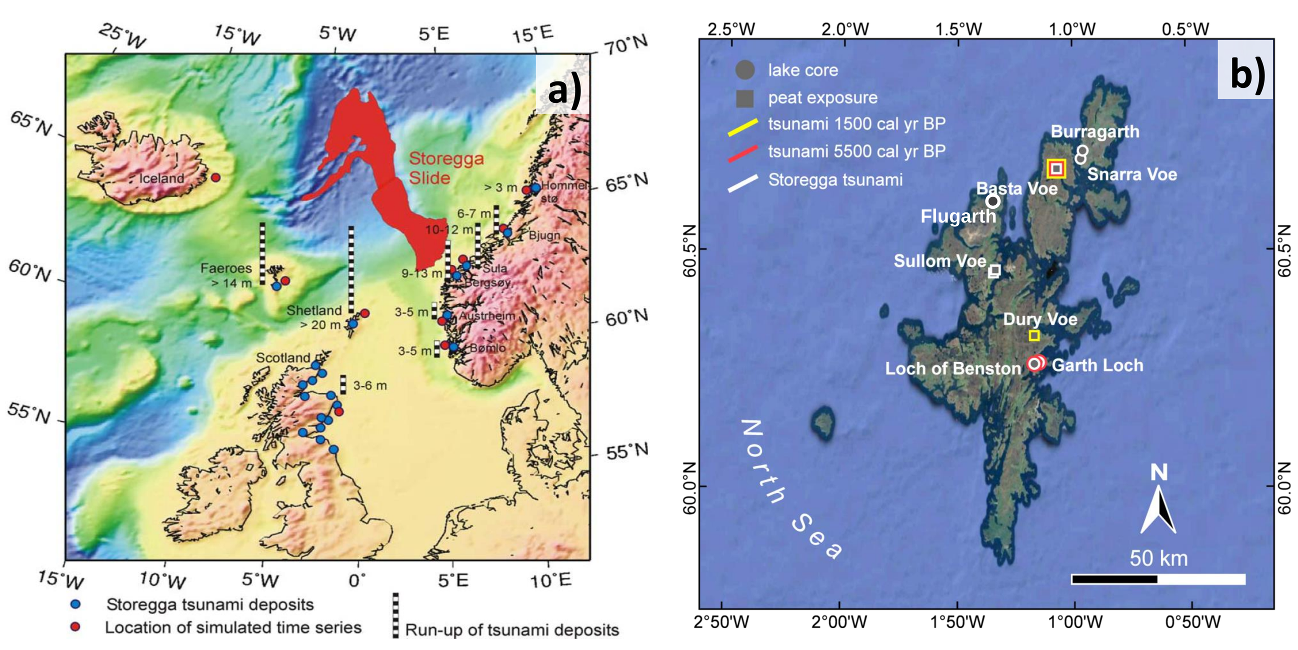
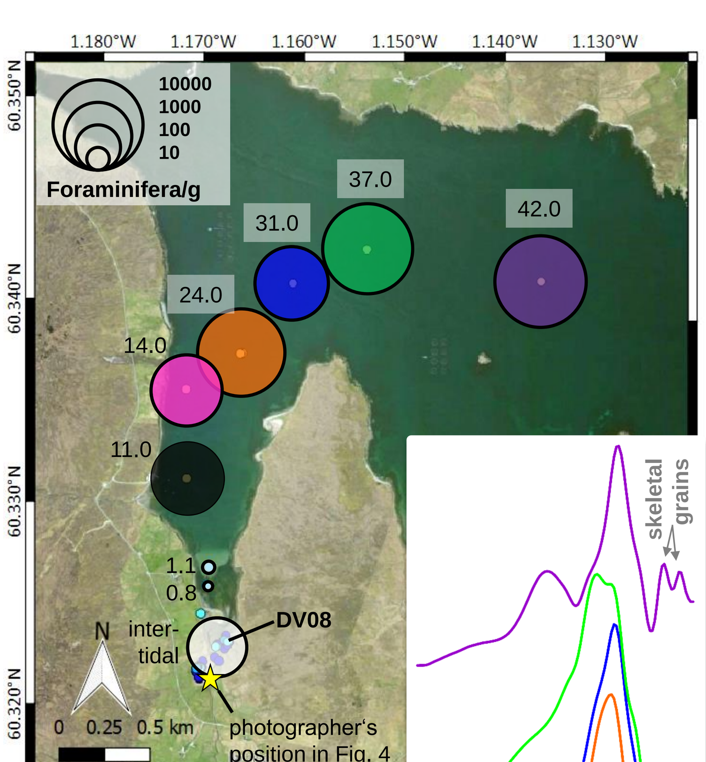


Fig. 2: a) Location of the main Storegga slide as well as correlating debris fans between Iceland, Scotland and Norway and tsunami deposits (Bondevik et al., 2005b); b) Field sites on Shetland with details on tsunami deposit occurrence. Evidence currently indicates three major events ~8150 (Storegga), ~5500 and ~1500 cal yrs BP (based on Bondevik et al., 2005a). Field sites in GEN-EX include Dury Voe, Garth Loch, Sullom Voe and Flugarth.

Study area and preliminary results:

- Shetland Islands – exposed to repeated tsunami impact (e.g. 8.15 ka Storegga slides → Fig. 2a; 5.5 ka; 1.5 ka).
- Distinct tsunami sand layers in coastal lowlands, e.g. at Dury Voe, 1.5 ka (Bondevik et al., 2005a) (Figs. 2b,3,6).
- Sampling campaign for eDNA study in March-April 2018 **(step 1 in Fig. 1)**: **Tsunami deposits** from onshore peat exposures (Dury Voe, Sullom Voe), coastal lakes (Flugarth, Garth Loch); modern source environments (Dury Voe).
- **No foraminiferal tests or skeletal grains found in palaeo-tsunami deposits → dissolved by low pH environment?**
- **Moderate to high foraminiferal concentrations and skeletal grains in inter-/subtidal samples (Figs. 3–5) → the main source area of onshore tsunami deposits.**
- **Successful DNA extraction from modern Foraminifera and palaeo-sediments (step 2, Fig. 1).**
- **Extensive 18S primer testing and polymerase chain reaction (PCR) optimisation** (using Phusion Hi-Fidelity polymerase + Dimethyl sulfoxide (DMSO) + Bovine Serum Albumin (BSA) to increase PCR yields) **(step 3 in Fig. 1)**.
- **Successful Sanger sequencing of foraminiferal DNA from modern offshore individuals.**
- **A-specific amplification of non-target (more dominant) DNA of other marine taxa in the palaeo-samples.**
- **Imminent: pilot test using shotgun metagenomic sequencing → to also detect non-dominant foram DNA in the palaeo-samples (step 4 in Fig. 1).**



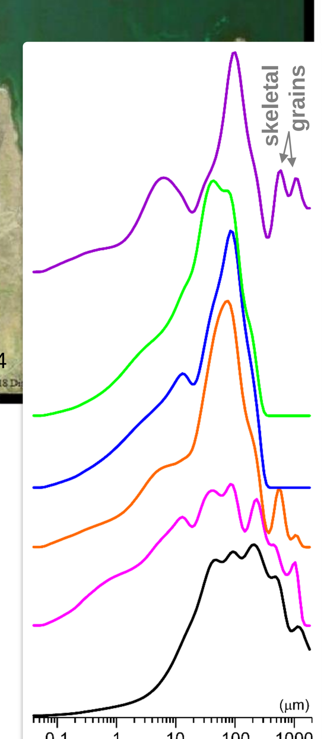


Fig. 3: Modern inter-/subtidal sampling sites at Dury Voe (Fig. 2b), with depths (m), foraminiferal concentrations and grain size data.

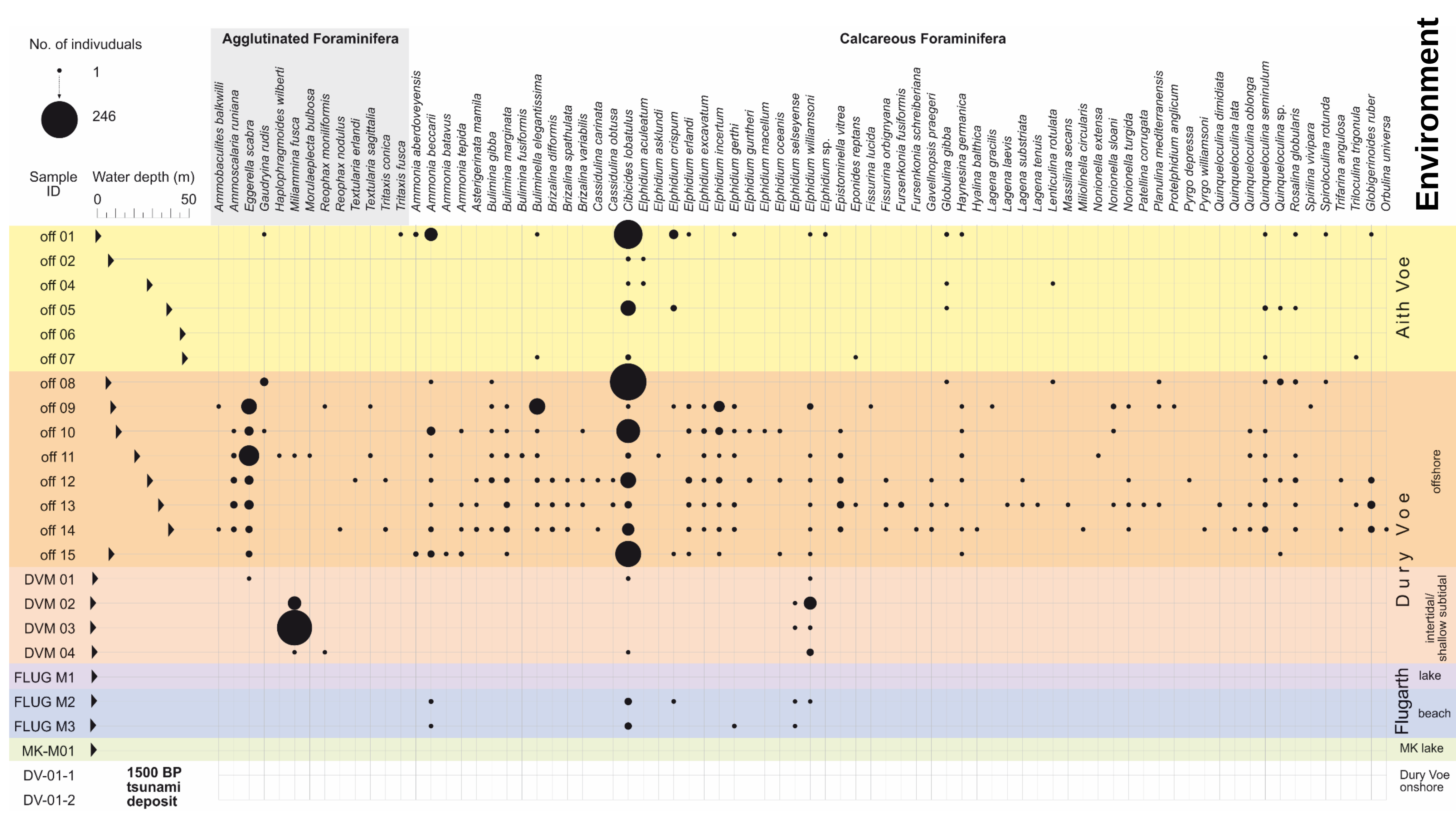


Fig. 4: The diverse foraminiferal record of modern intertidal and offshore environments of Shetland, representing the most likely sources of tsunami deposits. No forams found in tsunami deposits of DV 01. For location of sites, see Fig. 2b.

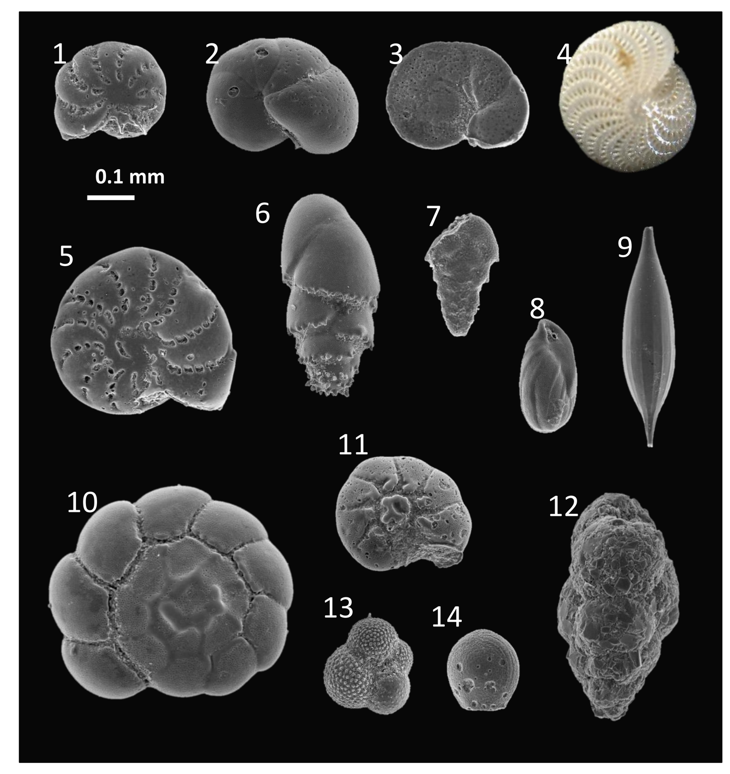


Fig. 5: Foraminifera of littoral environments and potential tsunami sediment sources of Shetland (Fig. 3). 1) Haynesina germanica; 2) Cibicides lobatulus (dorsal); 3) C. lobatulus (ventral); 4) Elphidium crispum; 5) Elphidium williamsoni; 6) Bulimina marginata; 7) Bryzalina spathulata; 8) Buliminella elegantissima; 9) Lagena gracilis; 10) Ammonia becarii (ventral); 11) A. becarii (dorsal); 12) Egerella scabra; 13) Globigerinoides ruber; 14) Orbulina universa.

***Fig. 6**: Peat-covered coastal lowland at the inner part of Dury Voe (photographer's position=star in Fig. 3). A thin tsunami deposit dated to 1.5 cal kyrs BP within the peat extends for several hundred metres inland.*

Acknowledgements: Funding is provided by the BELSPO BRAIN-be pioneer grant (BR/175/PI/GEN-EX). Permissions for fieldwork and sampling were kindly granted by local land owners.

References: **Bondevik**, S., et al., 2005a. Quat. Sci. Rev., 1757–1775; **Bondevik**, S., et al., 2005b. Mar. Petrol. Geol. 22, 195–208; **Engel**, M., et al., 2016. Earth-Sci. Rev. 163, 260–296; **Engel**, M., et al., 2020. Palaeogenomics of tsunami deposits. In: Engel, M., et al., (Eds.), Geological Records of Tsunamis and Other Extreme-Wave Events. Elsevier; **Goff**, J., et al., 2012. Sediment. Geol. 243–244, 70–88; **Szczuciński**, W., et al., 2016. Mar. Geol. 381, 29–33; **Thomsen**, P.F., **Willerslev**, E., 2015. Biol. Conserv. 183, 4-18.

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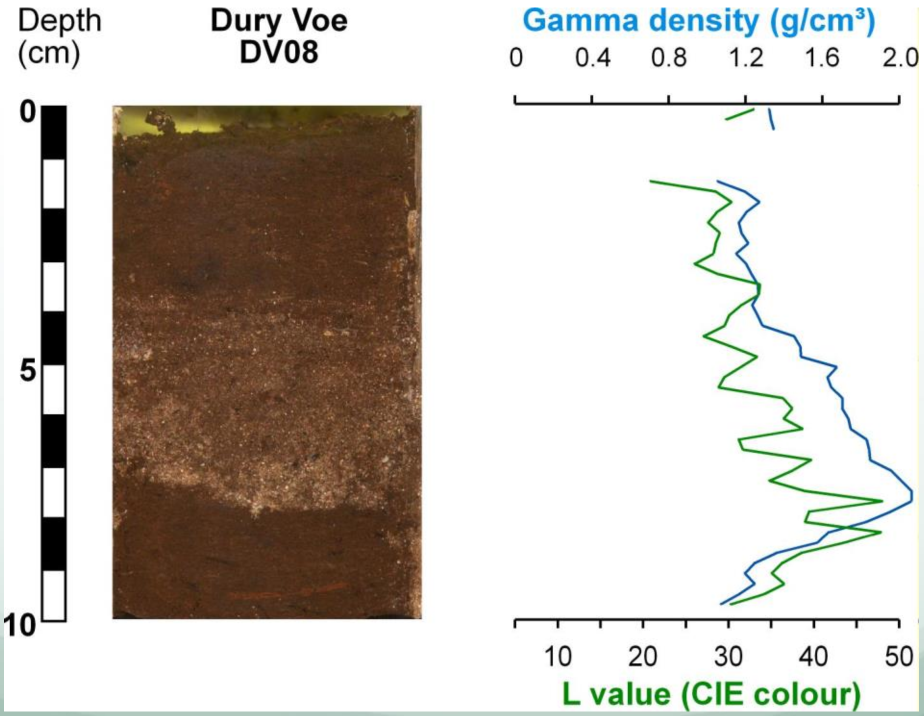
Sampling the tsunami layer at Dury Voe

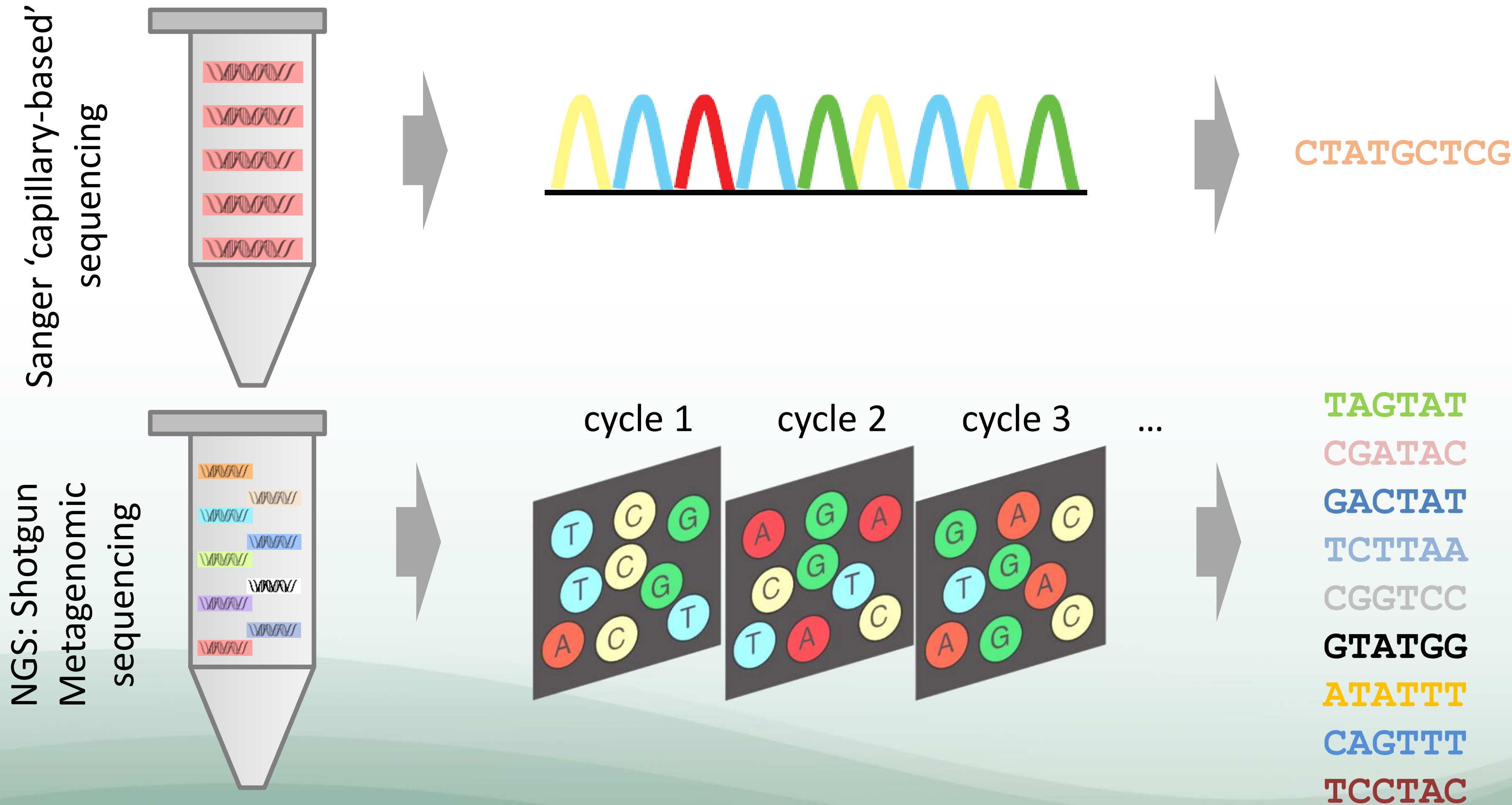
Geological Survey of Belgium

Profiles DV01 and DV08 at Dury Voe

Geological Survey of Belgium



Shotgun sequencing



Shendure and Ji 2008, Nature Biotechnology

Shotgun sequencing

Shotgun metagenomic sequencing:

- Enables sequencing of all organisms present within a DNA sample (i.e. the complex communities within tsunami sediments), even in those in low abundances (e.g. foraminifers)
- In contrast to capillary sequencing or PCR-based approaches, next-generation sequencing (NGS) allows sequencing in parallel
- Suitable for processing large batches of samples