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MICROPALAEONTOLOGICAL NOTEBOOK

How long after death is DNA preserved *in situ* in intertidal foraminifera?

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INTRODUCTION

Getting DNA from foraminifers is a difficult task, but it can greatly help the identification of species and the phylogeny of the group (e.g. Pawlowski, 2000; Schweizer *et al*., 2008; Pawlowski & Holzmann, 2014) which are still mainly based on test morphology. Therefore, it is crucial to document the test morphology of the sampled specimens and extract DNA from them separately from each other (Pawlowski & Holzmann, 2014). To increase the chances of recovering DNA from a single individual, it is best to pick live foraminifers. However, a major question is how long DNA can outlast an organism after its death in natural conditions (e.g. Lindahl, 1993). This question may seem trivial to biologists but many foraminiferologists with a geological background question the length of time of decay for DNA *in situ*. The preservation of DNA when storing foraminifers after sampling has been discussed previously (Holzmann & Pawlowski, 1996), but not its preservation *in situ* in intertidal conditions. The present experiment is designed to test how long after death DNA can still be obtained with routine DNA amplifications when picking dead foraminifers instead of living ones in intertidal environments.

MATERIAL AND METHODS

Sampling and picking of live specimens

Surface sediment was collected in an intertidal area of the Wadden Sea (Den Oever, The Netherlands, 52°56′24″N, 5°1′19″E), in May 2006 during the spring phytoplankton bloom. The sediment was sieved on a 63 µm screen, transferred to labelled plastic bottles with *in situ* seawater and stored in a fridge at 4 °C for up to several years (live specimens had survived to summer 2009). *Ammonia* Brünnich, 1772 were picked from the sediment and live individuals were distinguished from dead ones by natural coloration of the protoplasm and pseudopodial activity. Only live specimens were taken for further analyses. An individual from the same locality was sequenced for the partial SSU rRNA gene (GenBank accession number GQ853569) and identified as *Ammonia aomoriensis* (Asano, 1951) (Schweizer *et al*., 2011). This species is recognized as the phylotype T6 in molecular studies based on the partial LSU rRNA gene and has a disjunct distribution, known in Europe, China and Japan (Hayward *et al*., 2004). The specimens used in the present study probably also belong to *A. aomoriensis*.

Killing and DNA extraction

The live *Ammonia* were cleaned with a brush and placed in 1.5 ml plastic tubes with filtered seawater in a dry bath incubator at 60 °C for five minutes to be killed. This method aimed to kill most of the micro-organisms present in the samples without perturbing too much the biomolecules such as DNA and enzymes. Three *Ammonia* were immediately put in the extraction buffer, whereas the remaining ones were separated into two groups. Group W (wet) concerns specimens and seawater heated in the incubator and kept in tubes. We hypothesize that commensal micro-organisms present in the sample were also killed by the heat. Group S (sediment) concerns specimens heated in seawater and thereafter placed in Petri dishes with fine sediment (<63 µm) and filtered seawater which were not heated. In that case, micro-organisms present in the sediment and the water are still alive. Both groups were kept at room temperature (about 20 °C) until taken for DNA extraction. Three specimens of each group (W and S) were taken at hourly intervals after the killing for the first 12 h and thereafter every 12 h until 72 h for the W group and 24 h for the S group (Table 1). A second batch of live *Ammonia* was prepared in the same conditions as group W with five specimens taken every hour from $T=0$ to $T=6$ h (Table 2). Each specimen was extracted individually for DNA with the DOC buffer (buffer and method detailed in Pawlowski, 2000).

Table 1. Results of DNA amplifications for the three replicates of groups W (W1–W3) and S (S1–S3) sampled after death from T = 0 to T = 72 h.

T (h)	W1	W2	W3	S1	S2	S3
0	OK	OK	OK			
1	OK	negative	OK	OK	OK	OK
2	negative	negative	OK	OK	OK	OK
3	OK	negative	negative	negative	negative	negative
4	negative	negative	OK	OK	negative	negative
5	negative	OK	negative	negative	negative	OK
6	negative	negative	negative	negative	negative	negative
7	negative	negative	negative	negative	negative	negative
8	negative	negative	negative	negative	negative	negative
9	negative	negative	negative	negative	negative	negative
10	negative	negative	negative	negative	negative	negative
11	negative	negative	negative	negative	negative	negative
12	negative	negative	negative	negative	negative	negative
24	negative	negative	negative	negative	negative	negative
36	negative	negative	negative			
48	negative	negative	negative			
60	negative	negative	negative			
72	negative	negative	negative			

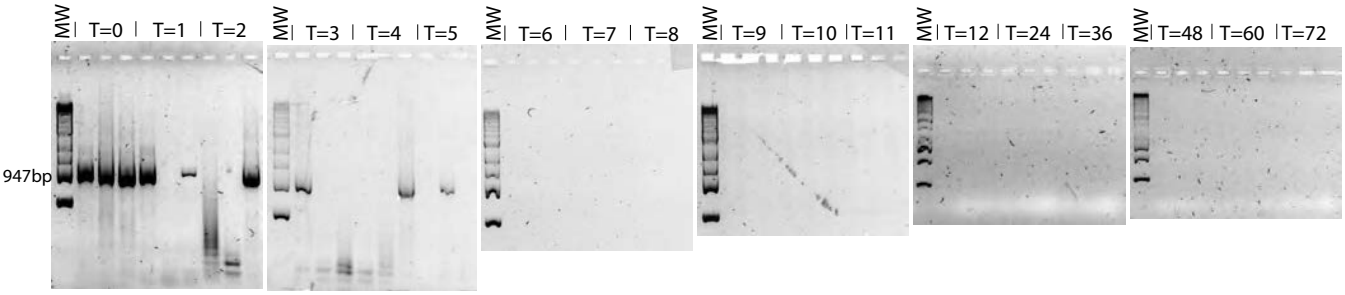

Fig. 1. Results of DNA amplifications for Group W individuals. Three individuals were tested for each time, from $T=0$ to $T=72$ h. Molecular weight (MW) indicated with the DNA marker Lambda DNA/EcoRI+HindIII Marker 3 (Fermentas). The amplicons have the same size as the third smaller band of the DNA marker, which is 947 base pair (bp) and is not separated from the second band (831 bp) in some of the gels.

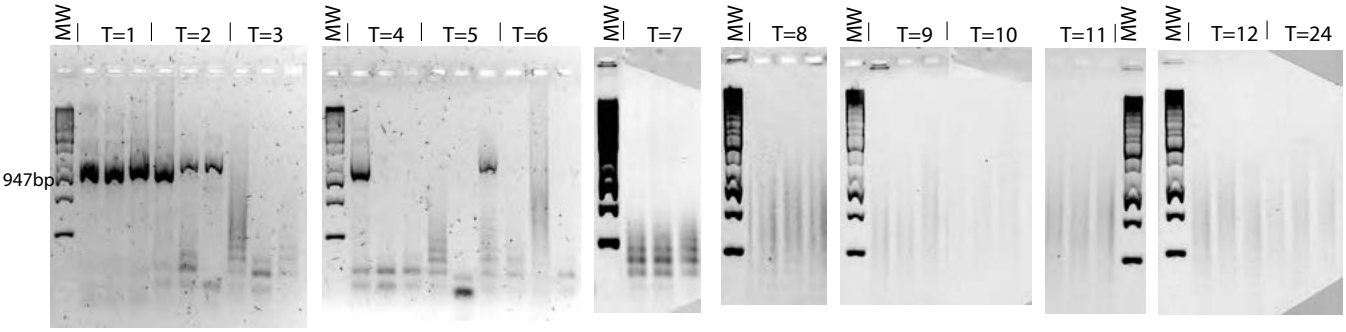

Fig. 2. Results of DNA amplifications for Group S individuals. Three individuals were tested for each time, from $T=1$ h to $T=24$ h. Molecular weight (MW) indicated with the DNA marker Lambda DNA/EcoRI+HindIII Marker 3 (Fermentas). The amplicons have the same size as the third smaller band of the DNA marker, which is 947 base pair (bp).

Table 2. Results of DNA amplifications for the five replicates of the second batch sampled after death from T=0 to T=6 h.

T (h)	a	b	c	d	e
0	OK	OK	negative	negative	OK
1	negative	negative	negative	OK	negative
2	negative	OK	negative	negative	negative
3	negative	negative	OK	negative	OK
4	negative	negative	negative	negative	negative
5	negative	negative	negative	negative	negative
6	negative	negative	OK	negative	negative

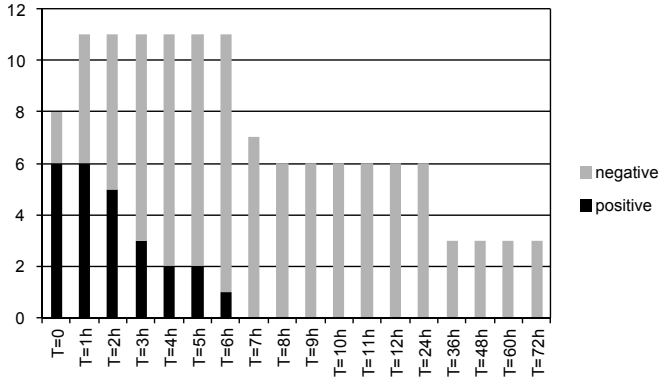

Fig. 3. Histogram showing the number of individuals for the different times after death ($T=0$ to $T=72$ h), with black denoting positive individuals and grey denoting negative individuals.

Amplification of DNA

The DNA amplification target was a fragment of the SSU rDNA, which is routinely used in foraminiferal molecular phylogenies and barcoding (Pawlowski, 2000; Pawlowski & Holzmann, 2014). The amplification was performed as described in Schweizer *et al*. (2005), with taxon-specific primers to ascertain the foraminiferal origin of amplicons. The results were checked on 1% agarose electrophoresis gels loaded with 4 µl of each sample and stained with GelRed (Biotium).

RESULTS AND DISCUSSION

The results of DNA amplifications are shown in Figures 1 and 2 and summarized in Tables 1 and 2. The amplification of shorter fragments may give better results, but our aim was to test the method routinely used for foraminiferal DNA and identify the effects of picking dead specimens. There is no significant difference between groups W and S (Table 1), which could be an indication that the DNA decay happens mainly through processes led by enzymes and other intracellular components in the first hours after death (instead of degradation by micro-organisms). This experience shows that the decay of DNA is relatively fast at room temperature, as within less than 7 h, all the samples have negative results for the amplification (Fig. 3). These results show the speed of the process under conditions of temperature and oxygenation, which can be found in intertidal areas with temperate climates. With lower temperatures and/or lower concentrations of oxygen, it is expected that the decay process would slow

down as shown by a recent study dealing with ancient DNA in the deep sea (Lejzerowicz *et al*., 2013). Therefore, because DNA decays within hours after death in temperate intertidal environments, it is extremely important to check that collected foraminifers are alive prior to the DNA extraction to obtain positive DNA amplification.

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REFERENCES

Hayward, B.W., Holzmann, M., Grenfell, H.R. & Pawlowski, J. 2004. Morphological distinction of molecular types in *Ammonia* – towards a taxonomic revision of the world's most common and misidentified foraminiferal genus. *Marine Micropaleontology*, **50**: 237–271, http://doi.org/10.1016/S0377-8398(03)00074-4

Holzmann, M. & Pawlowski, J. 1996. Preservation of foraminifera for DNA extraction and PCR amplification. *Journal of Foraminiferal Research*, **26**, 264–226, http://doi.org/10.2113/gsjfr.26.3.264

Lejzerowicz, F., Esling, P. *et al*. 2013. Ancient DNA complements microfossil record in deep-sea subsurface sediments. *Biology letters*, **9**: 20130283, http://doi.org/10.1098/rsbl.2013.0283

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

Pawlowski, J. 2000. Introduction to the molecular systematics of foraminifera. *Micropaleontology*, **46**: 1–12.

Pawlowski, J. & Holzmann, M. 2014. A plea for DNA barcoding of foraminifera. *Journal of Foraminiferal Research*, **44**: 62–67, http://doi.org/10.2113/gsjfr.44.1.62

Schweizer, M., Pawlowski, J., Duijnstee, I.A.P., Kouwenhoven, T.J. & van der Zwaan, G.J. 2005. Molecular phylogeny of the foraminiferal genus Uvigerina based on ribosomal DNA sequences. *Marine Micropaleontology*, **57**: 51–67, http://doi.org/10.1016/j.marmicro.2005.07.001

Schweizer, M., Pawlowski, J., Kouwenhoven, T.J., Guiard, J. & van der Zwaan, B. 2008. Molecular phylogeny of Rotaliida (Foraminifera) based on complete small subunit rDNA sequences. *Marine Micropaleontology*, **66**: 233–246, http://doi.org/10.1016/j.marmicro.2007.10.003

Schweizer, M., Polovodova, I., Nikulina, A. & Schönfeld, J. 2011. Molecular identification of *Ammonia* and *Elphidium* species (Foraminifera, Rotaliida) from the Kiel Fjord (SW Baltic Sea) with rDNA sequences. *Helgoland Marine Research*, **65**: 1–10, http://doi.org/10.1007/s10152-010-0194-3

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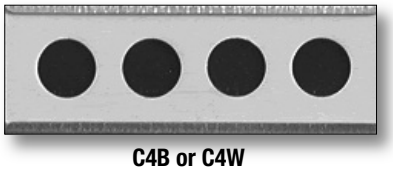

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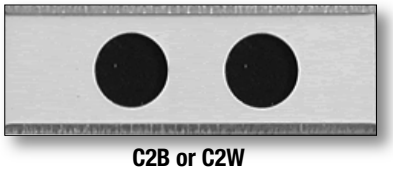

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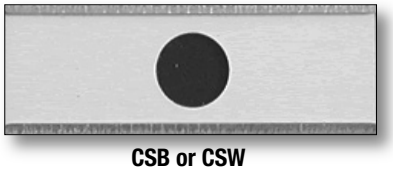

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