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6/03/2019

MASTS small grant scheme 2018. SG457: £500 to cover the cost of sectioning and transmission electron microscope (TEM) time at Edinburgh University.

Microbiome profiling of planktonic foraminifera: understanding ecological dependencies in a changing climate.

Aim

The aim of this project is to understand the ecology of planktonic foraminifera (calcifying protists) from transitional, subpolar and polar waters. Planktonic foraminifera in these habitats are currently most at risk to dissolution, due to the greater solubility of CO_2 in their colder waters and the subsequent shifts in pH and calcite saturation depth. Using transmission electron microscopy (TEM) coupled with 16S and 18S metabarcoding of the internal microbiome of planktonic foraminifera I will evaluate their trophic interactions, symbiotic associations, and their vulnerability to the changing climate.

Sample collection

Planktonic foraminifera of the species *Neogloboquadrina pachyderma* were collected from six stations in the Davis Strait (Baffin Bay, between Canada and Greenland, Figure 1) by vertical plankton net tows. Samples were immediately picked and transferred to fixative (4% paraformaldehyde and 2% glutaraldehyde in salt-adjusted phosphate buffered saline) for onward processing and sectioning before TEM analysis.

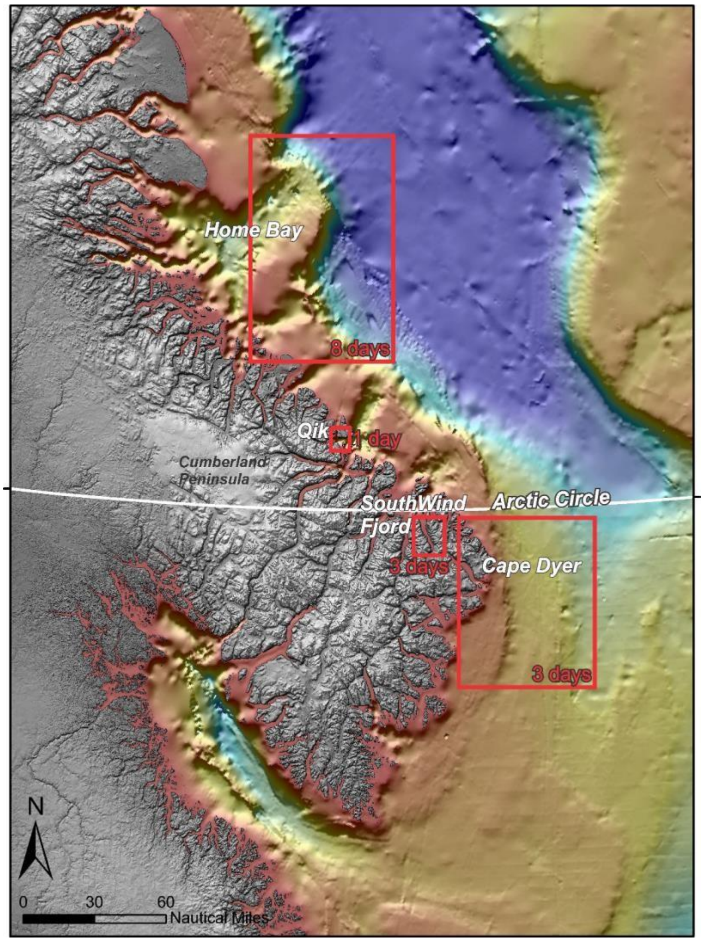

Figure 1. Map of the Davis Strait areas sampled by collaborators on board CCGS Hudson 2018042. Red rectangles show the areas within which sampling took place. Foraminifera for TEM analysis were collected from two stations from Cape Dyer, one from SouthWind Fjord and three from Home Bay.

Results and discussion

Fifteen foraminiferal samples were imaged via TEM. Four of the fifteen have provided poor micrographs that may be due to poor fixation or to diffuse cytoplasm as a result of the ontogenetic stage of the foraminifera at the point of collection. Further sectioning of these samples will be undertaken to establish this.

Eleven samples are of good quality. The images show the chambers of *Neogloboquadrina pachyderma* containing densely packed chloroplasts throughout the endoplasm (Fig. 2a), comparable with kleptoplastic benthic foraminifera such as *Haynesina germanica* (genetic type S16) and *Elphidium oceanense* (genetic type S4) (Darling et al., 2016; Jauffrais et al., 2018).

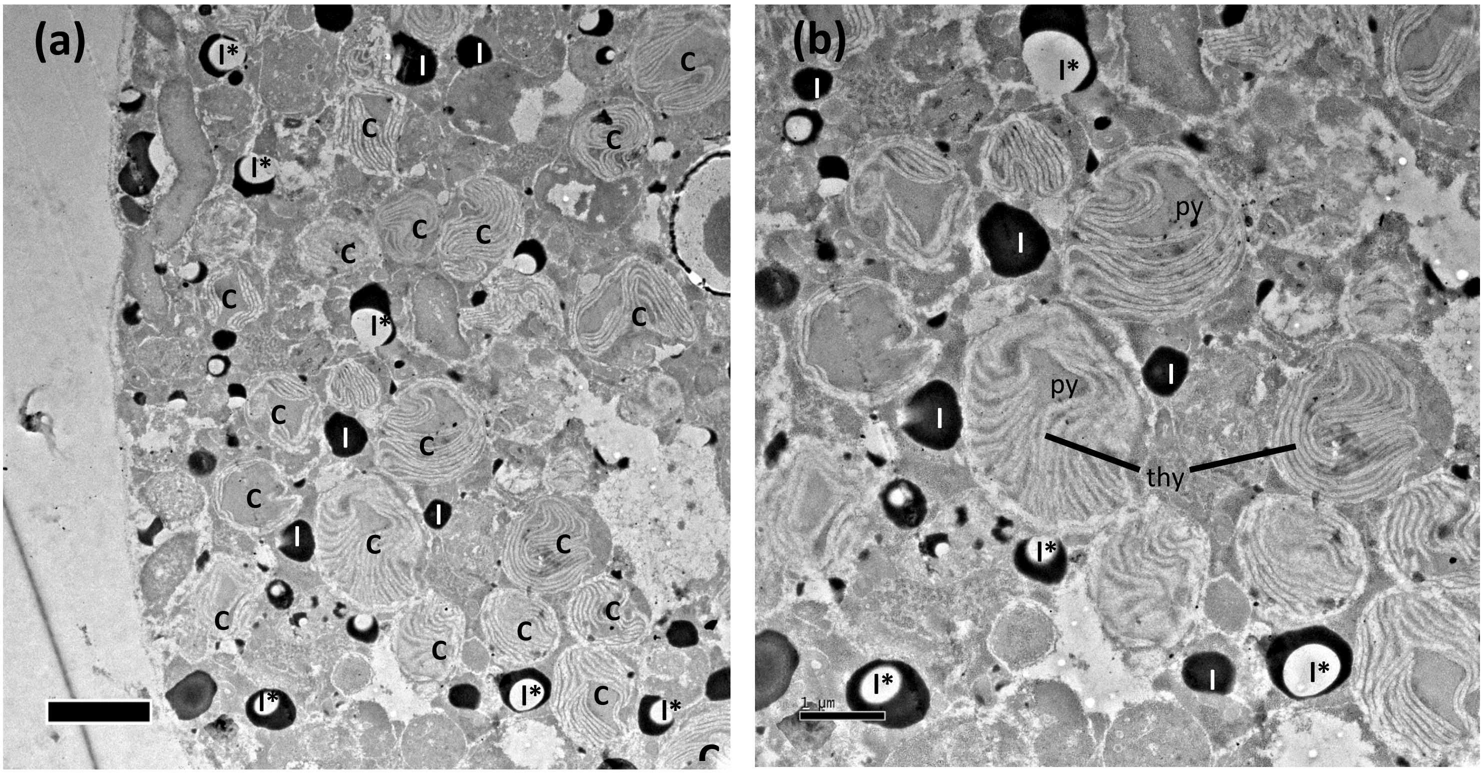

Figure 2. Transmission electron micrographs of *Neogloboquadrina pachyderma*. (a) Organisation of densely packed chloroplasts, c, within the cytoplasm, along with lipid droplets, l, and lipid droplets in degradation, l*. (b) The pyrenoid, py, and the thylakoid membranes, thy, can be observed in the chloroplasts. Scale bar (a) 2µm (b) 1µm

Whilst algal symbionts are widely reported in planktonic foraminifera, Kleptoplasty has never been reported. Benthic foraminifera have been shown to harbour chloroplasts in many species (Jauffrais et al., 2018), but with differing strategies. Some species harbour chloroplasts that are found around the periphery of the cell (e.g. *Elphidium williamsoni* (S1), *E. selseyense* (S7); *Planoglabratella opercularis*; Jauffrais et al., 2018) whilst others like *N. pachyderma* reported here, contain chloroplasts that are evenly distributed throughout the cell endoplasm (*E. oceanense* (S4), *E. crispum* (S11); Jauffrais et al., 2018). To date only *H. germanica* with its evenly distributed chloroplasts, has been proven to assimilate fixed carbon (LeKieffre et al., 2018), so containing and maintaining a population of functional chloroplasts. Although this must be a primary reason for sequestering chloroplasts, *Nonionellina labradorica* that lives below the photic zone, also sequesters chloroplasts from the cosmopolitan planktonic diatom *Thalasossira.* The functionality of these chloroplasts is unknown as they do not fix carbon, and do not appear to be involved in nitrogen or sulphur metabolism (Jauffrais et al., 2019). Kleptoplasty remains a poorly understood function in foraminifera, and its occurrence in planktonic foraminifera is a novel finding that requires further study.

In addition to chloroplasts, diatoms and empty diatom frustules were observed inside the foraminiferal cytoplasm (Fig. 3). This indicates that diatoms are a food source for *N. pachyderma* and, like in benthic foraminifera, are probably the source of the densely packed chloroplasts. A diatom diet agrees with previous reports that non-spinose planktonic foraminifera have an herbivorous diet, and are observed at the chlorophyll maximum (Hemleben et al., 1989). Our plankton tows were vertical net tows from 100m, so the foraminifera sampled were dwelling in the photic zone at the time of collection. Metabarcoding analysis will provide evidence of the source of the chloroplasts and diet of the foraminifera.

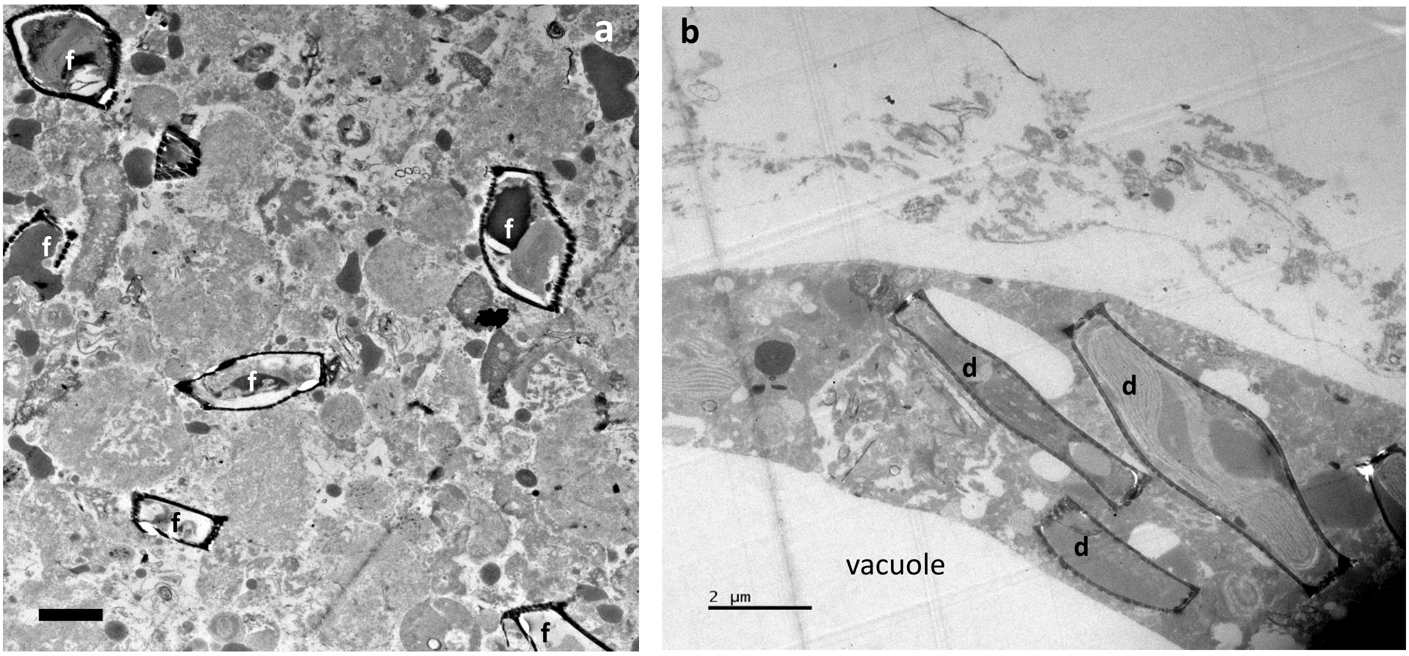

Figure 3. Transmission electron micrographs of diatoms and diatom frustules within *Neogloboquadrina pachyderma*. (a) Empty frustules/degrading diatom cells, f, within the enoplasm. (b) Ingested diatoms close to cell surface, still containing intact diatom cells, d. The chloroplasts can be seen clearly.

Summary and further questions

This is the first report of kleptoplasty in the planktonic formainifera. The implications of this for understanding the potential for *N. pachyderma* to survive climate changes need to be investigated. It has been reported that mixotrophic foraminifera (species with more than one feeding strategy) have a greater potential for adaptation and survival as temperatures warm (Roy et al., 2015), and indeed mixotrophic organisms dominate in oligotrophic regions (e.g. Hartman et al., 2012). Would *N. pachyderma* therefore have a greater adaptation potential than expected due to a combination of kleptoplasty and heterotrophy as feeding strategies?

If a particular species of diatom provides the main bulk of food and kleptoplasts to *N. pachyderma,* what are the consequences of a diatom population shift in the Arctic due to ocean warming? In addition, photosynthesis by algal symbionts is known to affect shell geochemistry and creates the need for species-specific palaeotemperature equations. There is potential therefore for photosynthesising kleptoplasts to also affect shell chemistry. This is of significance because the more temperate dwelling *N. incompta* has only recently been identified as a separate species from *N. pachyderma* (Darling et al., 2006) and previously palaeotemperature equations have been base on *N. incompta* which is not kleptoplastic and does not contain algal symbionts (Bird et al., 2018).

Metabarcoding will help to shed further light on the diets and kleptoplast supplies to *N. pachyderma*. In addition, future studies using coupled TEM-NanoSIMS and genetic investigations would help elucidate the function of the sequestered chloroplasts.

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