Strain-specific calcification response of *Gephyrocapsa huxleyi* to pH change: COCCACE, a high-throughput live imaging method

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In *Gephyrocapsa huxleyi*, the calcification response to changing carbonate chemistry is believed to be strain-specific. In order to test this hypothesis, we developed a high-throughput live imaging method of microscopy, coupled with an experimental setup, that would be able to: 1) continuously estimate the calcification of the coccolithophores; 2) control and stabilise the pH and *p*CO; and 3) have numerous cultures in the same batch. We used an automated inverted microscope, a light wavelength of 562 nm (that does not excite chlorophyll), a pair of circular polarisers, a 48-multiwell plate placed in an atmosphere-controlled chamber via a CO_2 mixer, and a numerical camera. A computer controlled this setup. Twenty-three strains of *G. huxleyi* were selected in order to represent a large panel of oceanographic conditions. Five pH experiments were made, ranging from 8.20 to 7.44. After being acclimated to the medium, two replicates of each of these strains were transferred into the wells for about 80 hours, with an alternating day/night of 12/12 hours. Twenty-four fields of view for each well were imaged every two hours. The pH was maintained by flushing with a $\%CO_2$ atmosphere that corresponded to the desired pH. The images were treated with software that measured the number of coccospheres in each well, their size, their area and the calcite mass. It was therefore possible to estimate instantaneous calcification rates. The reproducibility among the replicates was high. A circadian evolution of the calcification was observed in most cases. The optimum pH of calcification for each strain was evaluated. Four cases were observed – strains that calcify best at high, mid or low pH, and those that were not influenced by pH. This work illustrates the complexity of predicting the potential adaptation to future ocean acidification by *G. huxleyi*.