Response of *Ammonia confertitesta* (T6) to a triple stressors experiment

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Context

Ocean acidification, warmer temperatures, and the expansion of hypoxic zones in coastal areas are direct consequences of the increase in anthropogenic activities. However, the combined effects of these stressors on calcium carbonate-secreting marine microorganisms - foraminifera are complex and poorly understood.

This Master thesis aimed at understanding the effects of triple stressors (warming, acidification, deoxygenation) on foraminiferal species *Ammonia confertitesta* (T6) (Hayward et al., 2021) based on the IPCC SSP3-7.0 (+4 ˚C) scenario. Specimens were collected in the Gullmar Fjord (Figure 1) in September 2022.

The objectives were (1) to provide a stable experimental set-up with duplicates aquariums (2) to assess foraminiferal response (presence, survival, calcification) between conditions and duplicates (3) to investigate the trace elements incorporation (Mg, Mn, Ba, and Sr).

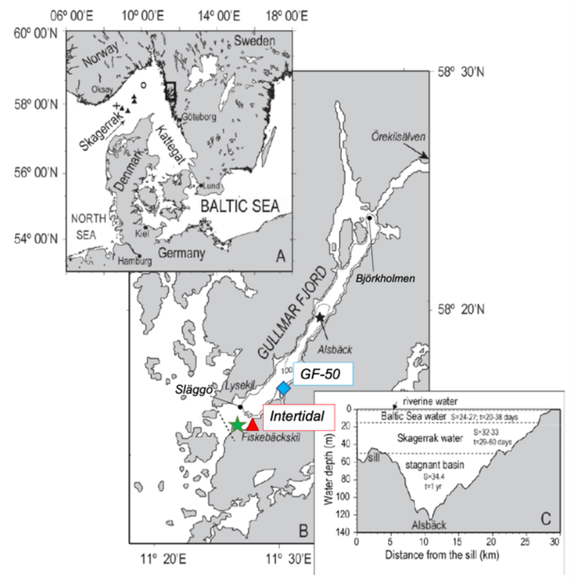


Figure 1: A. Geographic location of Gullmar fjord. B. Map of the fjord's studied station; blue diamond: GF50 oxic station; Red triangle: Intertidal station; Green star: Kristineberg Centre for Marine Research and Innovation; C. Hydrographic and topographic parameters (Modified from Choquel et al., 2021)

Take home messages

- → Stable experimental set-up for the 10 conditions
- → *A. confertitesta* calcified under one or two combined stressors, but not three combined stressors (Hypoxic, low pH, and warm) suggesting that the species may have reached its tolerance threshold to thrive.
- → A large variability in foraminiferal response (up to a factor of two) was observed between conditions and also between duplicates, which may imply an influence of internal factors (e.g., metabolism) rather than only external environmental factors.
- → A large variability of the TE/Ca ratios of foraminifera growing in low-pH environments may suggest a struggle of the species to calcify at equilibrium in these conditions.

Methods

1) Decoupled (T ˚C, pH, and O_2) experimental design. Two thermo-regulated rooms (T9 ˚C and T14 ˚C) displaying control (normal pH 8, oxic), medium pH (pH 7.6, oxic), and low pH (pH 7.4, oxic), reached through CO_2 and air bubbling. Additionally, hypoxic high-pH (pH 8.0, $[O_2] < 63$ µmol L^{-1}) reached through nitrogen bubbling, and combined effects of hypoxic + low-pH (pH 7.4, $[O_2] < 63$ µmol L^{-1}), reached through nitrogen and CO_2 bubbling. Regular measurements of the water parameters (T°C, salinity, alkalinity, pH, $[O_2]$)
2) Settlement of duplicate tanks for controls and low pH aquariums. The experimental setup is summarized in Figure 2
3) Calcein and Cell Tracker Blue (CTB) labeling to assess calcification and survival percentages
4) Laser Ablation-ICP-MS analyses on newly formed chambers for TE/Ca incorporation (Mg, Mn, Ba, and Sr)

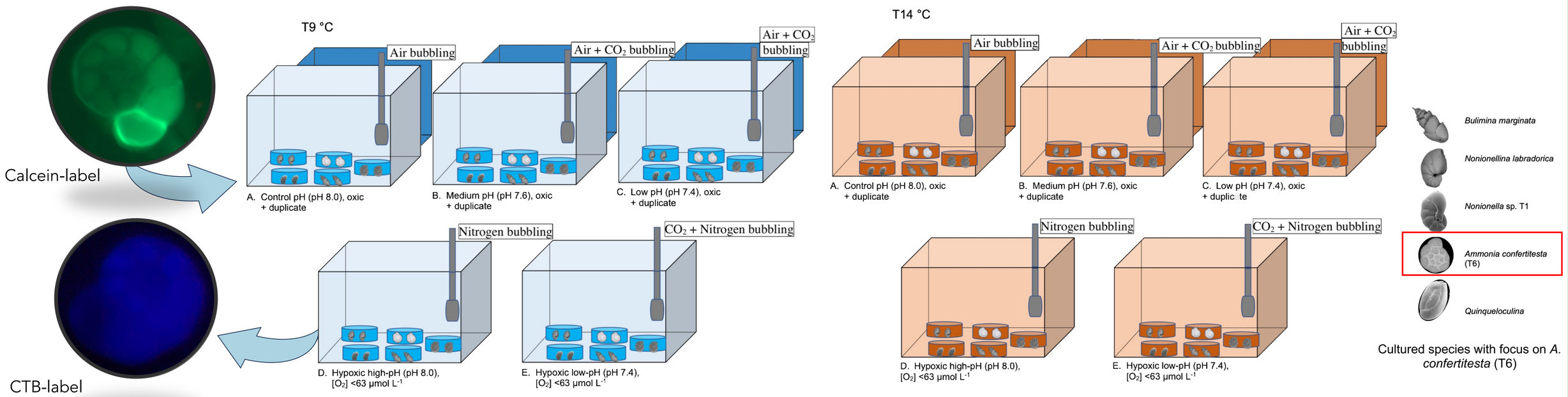


Figure 2: Experimental set-up in the cold room (T9˚C) and warm room (T14 ˚C)

Results and Discussion

Foraminiferal response

- → Survival in all experimental conditions.
- → Calcification in almost all conditions excepted in Hypoxic low-pH warm
- → Differences observed between duplicates, sometimes by factor 2

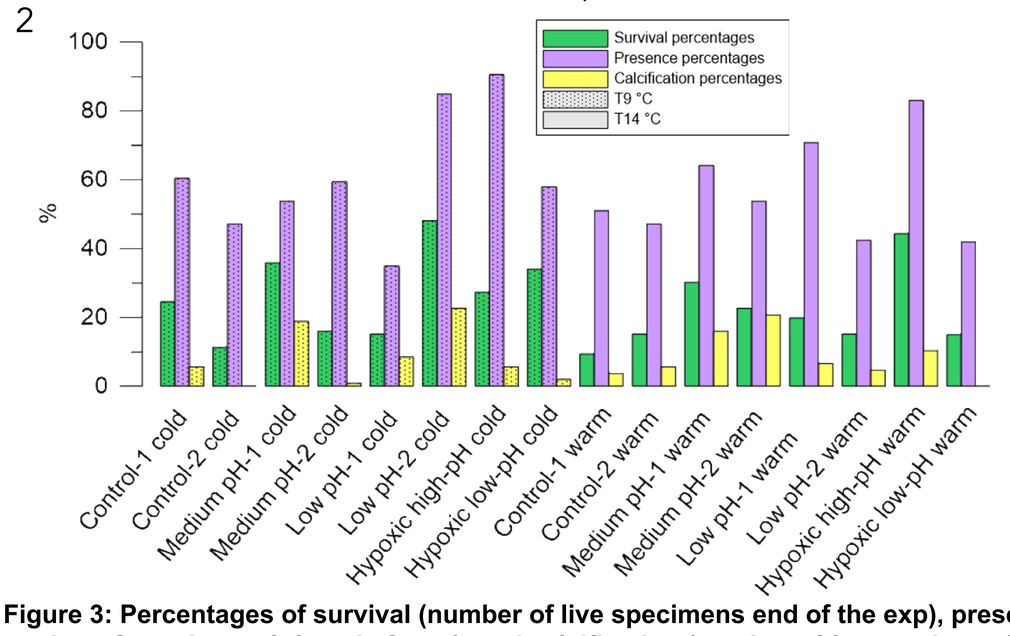
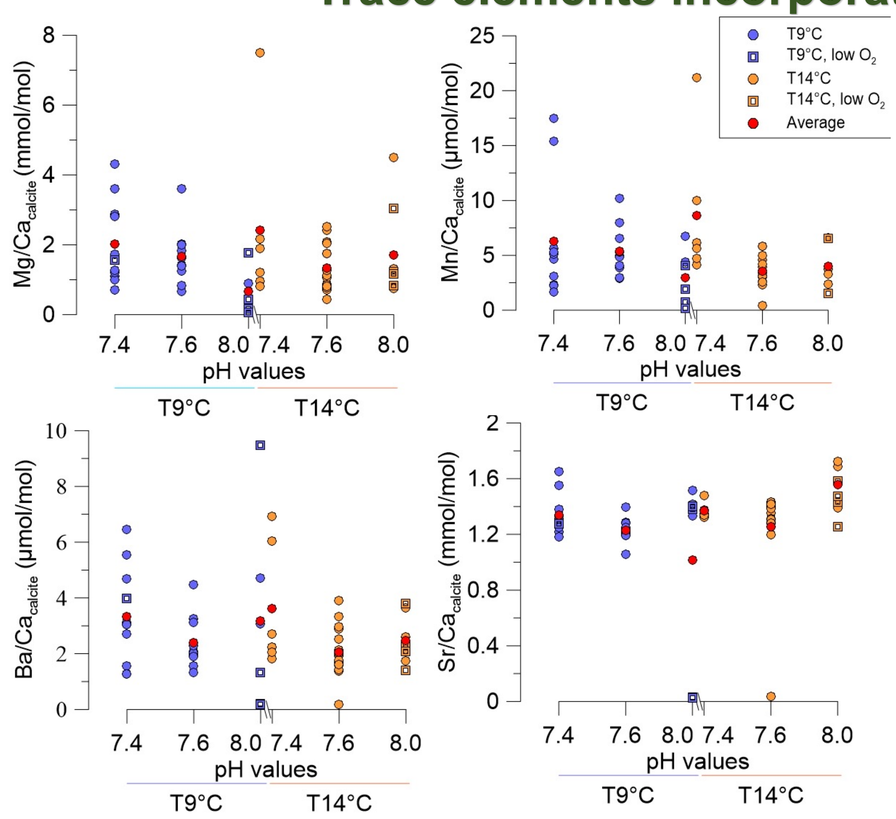


Figure 3: Percentages of survival (number of live specimens end of the exp), presence (number of specimens left end of exp.), and calcification (number of forams that calcified new chambers)

Trace elements incorporation



- → No ontogenetic effects between chamber stages, advantageous for environmental reconstruction.
- → Consistent values with comparable culturing experiments.
- → No trends in the TE incorporation.
- → More variability at low-pH for Mg, Mn, and Ba.
- → Sr generally stable.

Figure 4: Mg/Ca (mmol/mol), Ba/Ca (µmol/mol), and Mn/Ca (µmol/mol) and Sr/Ca (mmol/mol) according to pH and T°C

Reference:
Hayward et al., (2021). *Micropaleontology*; Choquel et al., (2021). *Biogeosciences*.

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