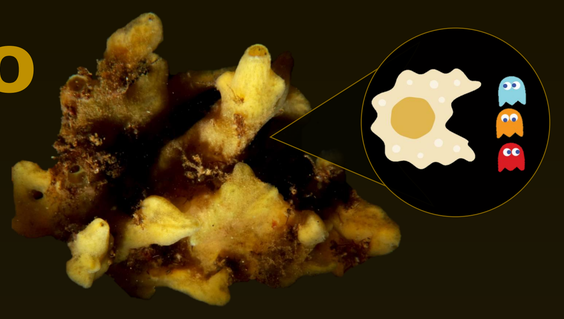
# Development of a novel, quantitative in-vivo phagocytosis assay to unravel mechanisms of interaction in sponge-microbe symbiosis



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## Background

Sponges harbor diverse, specific, and stable microbial communities, but at the same time, they efficiently feed on microbes from the surrounding seawater. How sponges discriminate between different microbes is still largely unknown and experimental assays are missing[1-6]. We hypothesize that phagocytosis by sponge cells is the key process controlling food digestion, symbiont colonization, and pathogen elimination. For testing this, we used the Baltic sponge *Halichondria panicea*, an emerging model organism to study symbiotic interactions.

## Aim

Develop an in-vivo assay to quantify phagocytosis of microbes by sponges

## Conclusions

- To our knowledge, this is the first quantitative phagocytosis in-vivo assay in sponges that can now be used to quantify the incorporation of microbes into sponge cells.
- Our method allowed us to identify differences in the phagocytosis process itself e.g., in the number of particles incorporated and degree of digestion by the sponge cells
- With our assay, we intend to query whether sponges process different microbes distinctively and unravel the role of phagocytosis sponge-microbe interactions.

## In-vivo assay experimental design

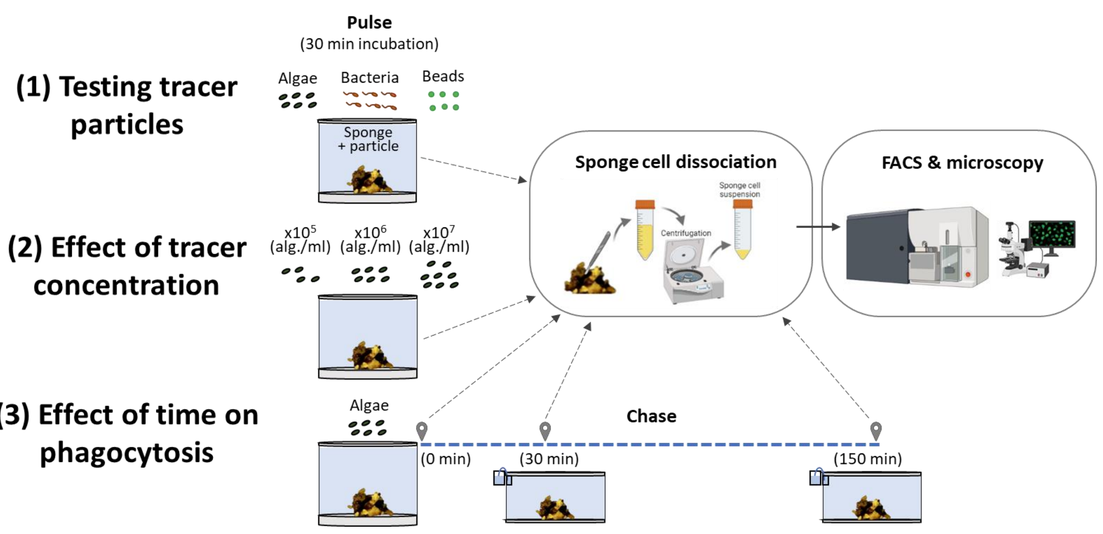


**Fig. 1.** Schematic representation of the in-vivo assay using different algae (*Nannochloropsis* sp.), TAMRA-stained bacteria (*Vibrio* sp.), and fluorescent latex beads (1 µm). Different algal concentrations and chase periods were tested. Sponge cells were dissociated, fixed, and used for FACS analysis and microscopy. (n = 4 per treatment)

## (1) Testing tracer particles

- The sponge removed 12-30% of the particles presented during the incubations.
- All particle types were incorporated into the sponge cells and successfully tracked.
- The average % of phagocytic cells was between ca. 5%-12%.
- The number of particles removed by the sponge was positively related to the cells' phagocytic activity for all tracers (Fig. 3).

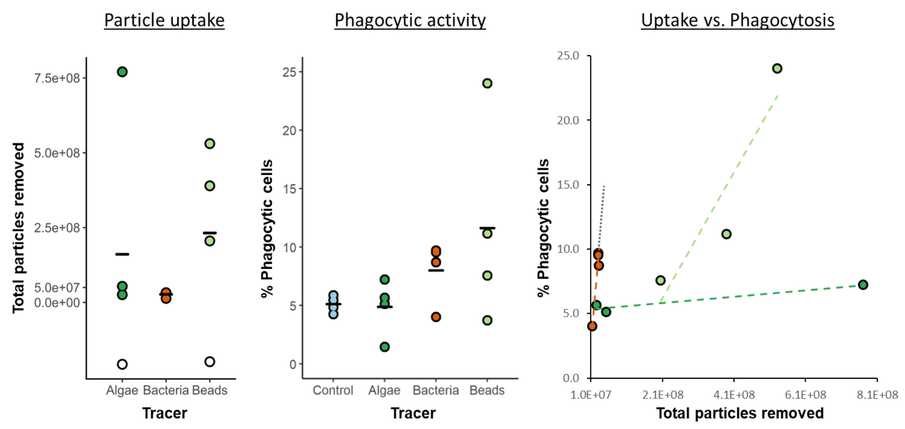


**Fig. 3.** Particle uptake and phagocytic activity of sponges incubated with different tracers. The phagocytic activity was not significantly different among particles (Kruskal-Wallis; p = 0.31). Unfilled circles: negative values not included in the uptake vs. phagocytosis comparison. Dotted gray line: extension of the slope calculated with the linear regression equation.

## Quantification of phagocytic cells with fluorescence-activated cell sorting (FACS)

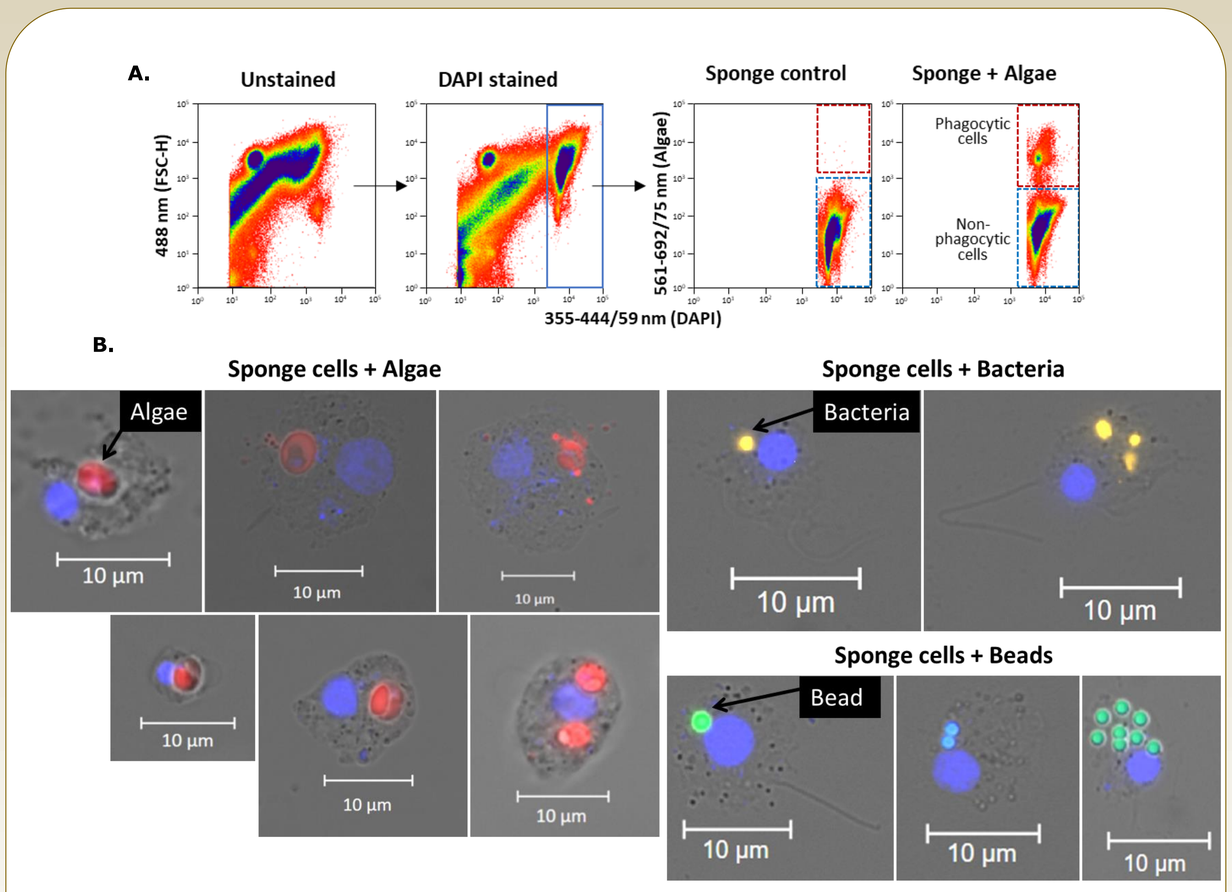


**Fig. 2. A.** Representative FACS cytograms showing gates for DAPI stained (blue rectangle), phagocytic and non-phagocytic cells (blue and red dashed rectangles, respectively). Control: sponge incubated without algae. **B.** Microscope pictures of sorted phagocytic cells. Sponge cell nuclei (blue) stained with DAPI.

## Effect of (2) tracer concentration and (3) time on phagocytosis

- The initial algal concentration did not significantly affect algal cell removal by the sponges.
- But, the phagocytic activity significantly increased with higher algal concentration (Fig 4A):
  - ca. 5-fold increase from $10^5$ to $10^6$ algae/ml.
  - ca. 2-fold increase from $10^6$ to $10^7$ algae/ml.
- Sponge cell phagocytosis is a fast process, occurring already within 30 min of exposure to the tracers.
- Phagocytic activity peaked at 0 min chase and afterwards significantly by ca. 50% (Fig 4B).

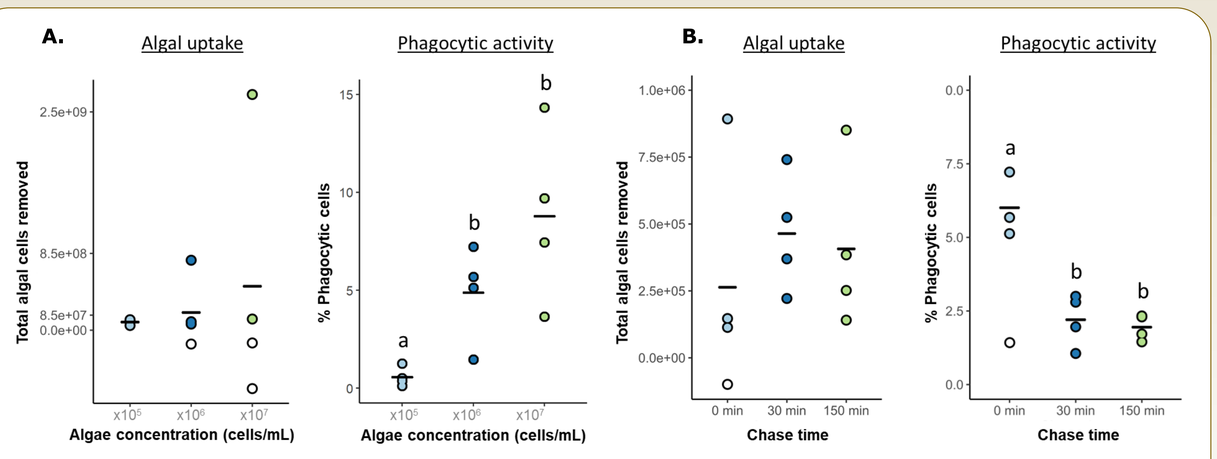


**Fig. 4.** Algal uptake and phagocytic activity of sponges **A.** incubated with different concentrations of algae and **B.** using different chase periods. Unfilled circles: negative values or outliers. Treatments marked with the same letter are not significantly different at α=0.05 (**A.** Kruskal-Wallis; p = 0.014 and **B.** one-way ANOVA; p < 0.001 ).

**Acknowledgments & funding sources:**
Marine Symbioses Unit Team
CRC 1182

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