A sequencing-based approximation to phytoflagellate bacterivory at individual resolution

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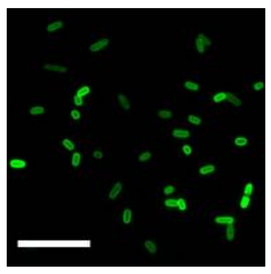
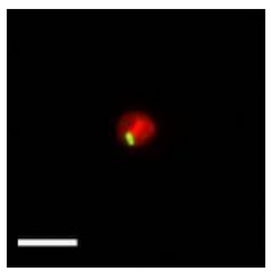
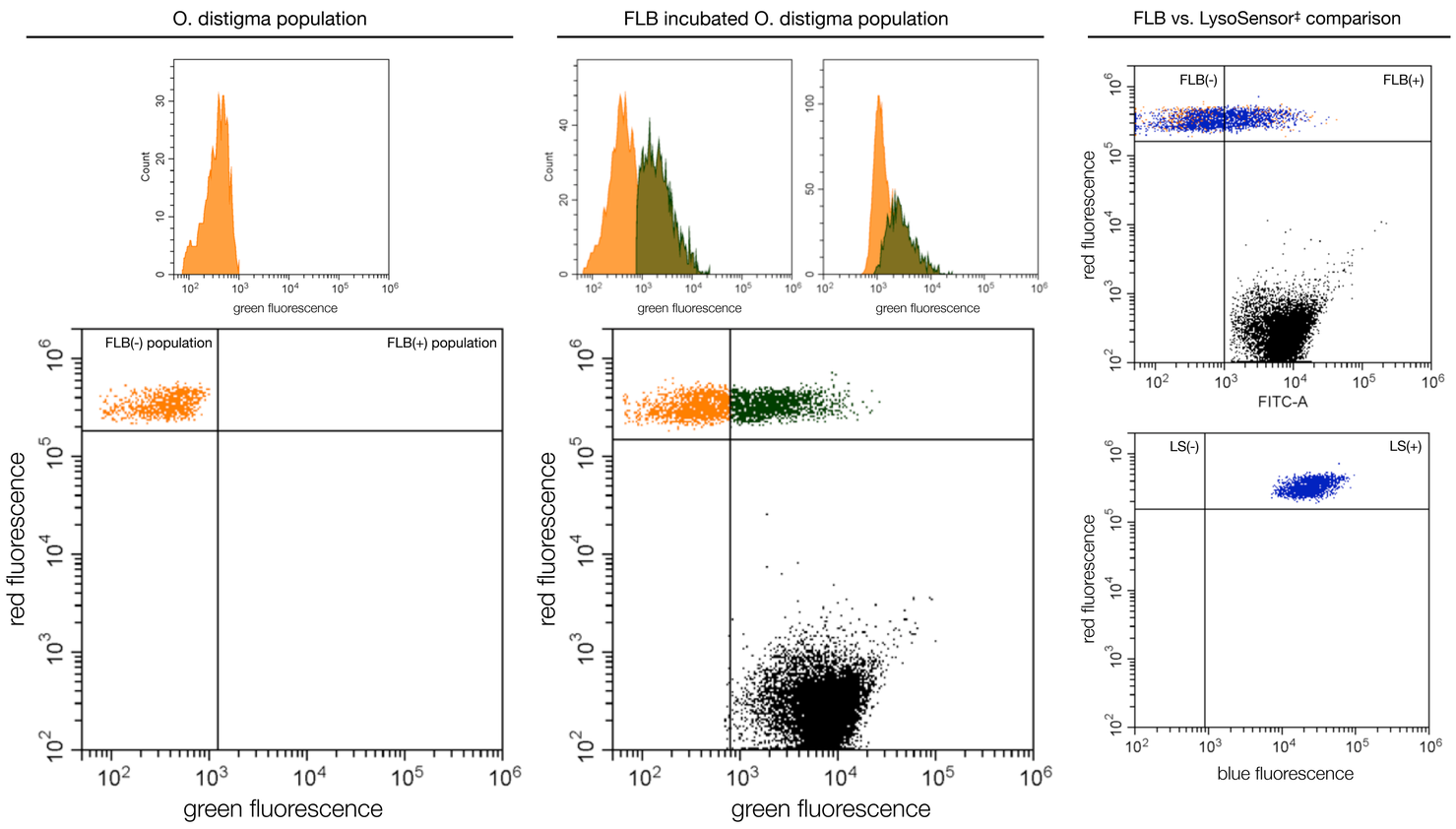
cytometry tests with cultured *Ochromonas distigma*, used as a model mixotrophic phytoflagellate



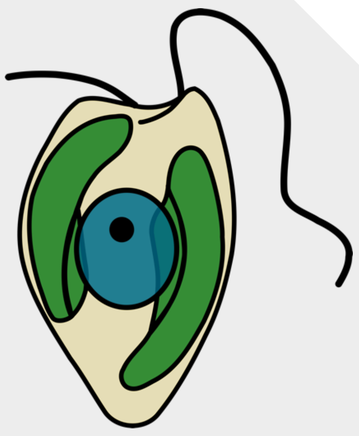
FLB preparation from *E. coli*.
Scale bar: 10 µm



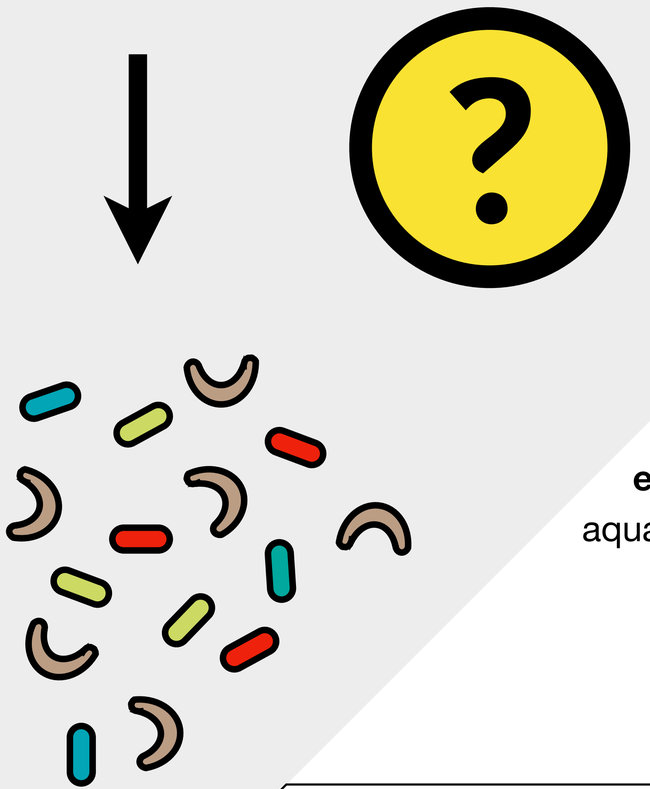
Sorted O. distigma cell from the FLB(+) population.
Scale bar: 10 µm



red: EX 405 EM 660/20 / green: EX 488 EM 525/40 / blue: EX 405 EM 450/45
‡LysoSensor DND-167, an acidotropic probe that stains food vacuoles and other acidic organelles, reported successful in retrieving actively grazing protists[2].



Mixotrophy is largely unexplored but increasingly recognised as a rule rather than an exception within eukaryotic phytoplankters[1]. We are testing the efficiency, sensitivity and selectivity of employing **fluorescently labelled bacteria** (FLB) ingestion coupled to flow cytometry aiming to isolate mixotrophs via **fluorescence activated cell sorting** (FACS), taking advantage of the phagocytised FLB signal together with their natural chlorophyll autofluorescence.

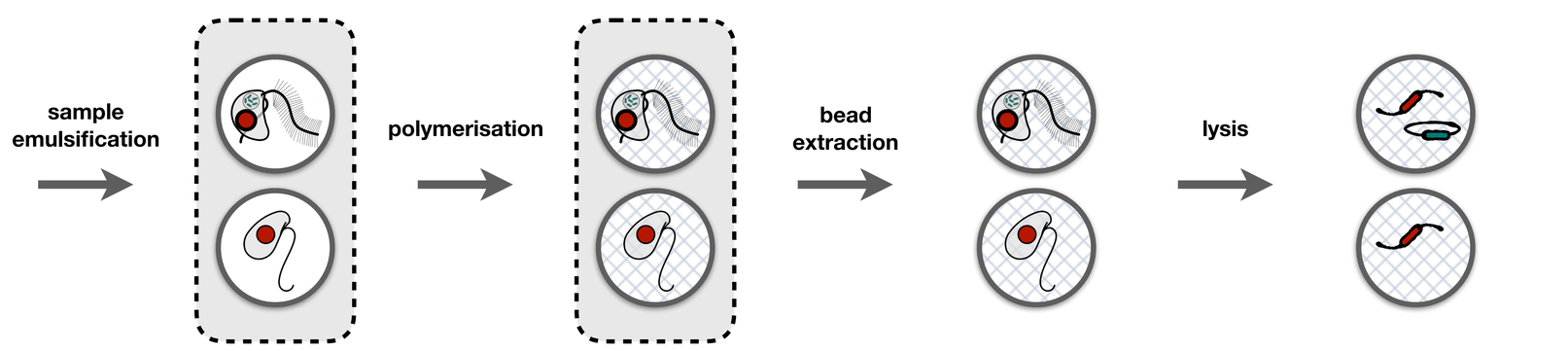


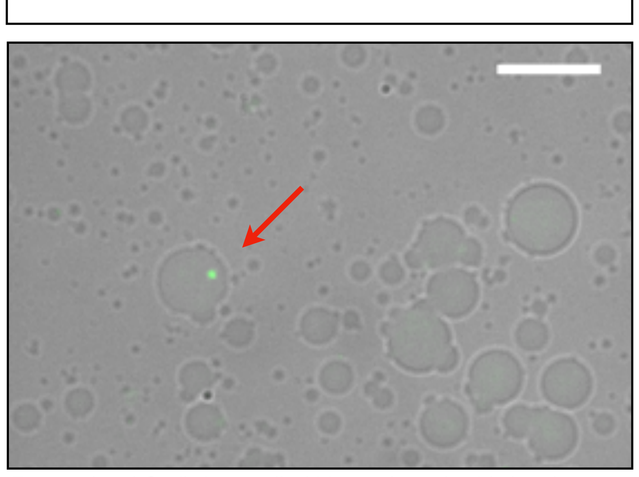
Who are they?

Who do they prey upon?

Current methods to individually **link prey to predator** at microscopic level, such as CARD-FISH, require previous knowledge of the interacting species and thus lack in exploratory power. We are implementing **emulsion, paired-isolation and concatenation PCR** (epicPCR)[3] to uncover bacteria-protist interactions in aquatic environments via concatenation of taxonomic markers.

ongoing encapsulation and amplification tests with cultured *O. distigma* fed with the flavobacterium *Dokdonia sp.*





Encapsulated *O. distigma* cell within a polyacrylamide bead.
Scale bar: 50 µm

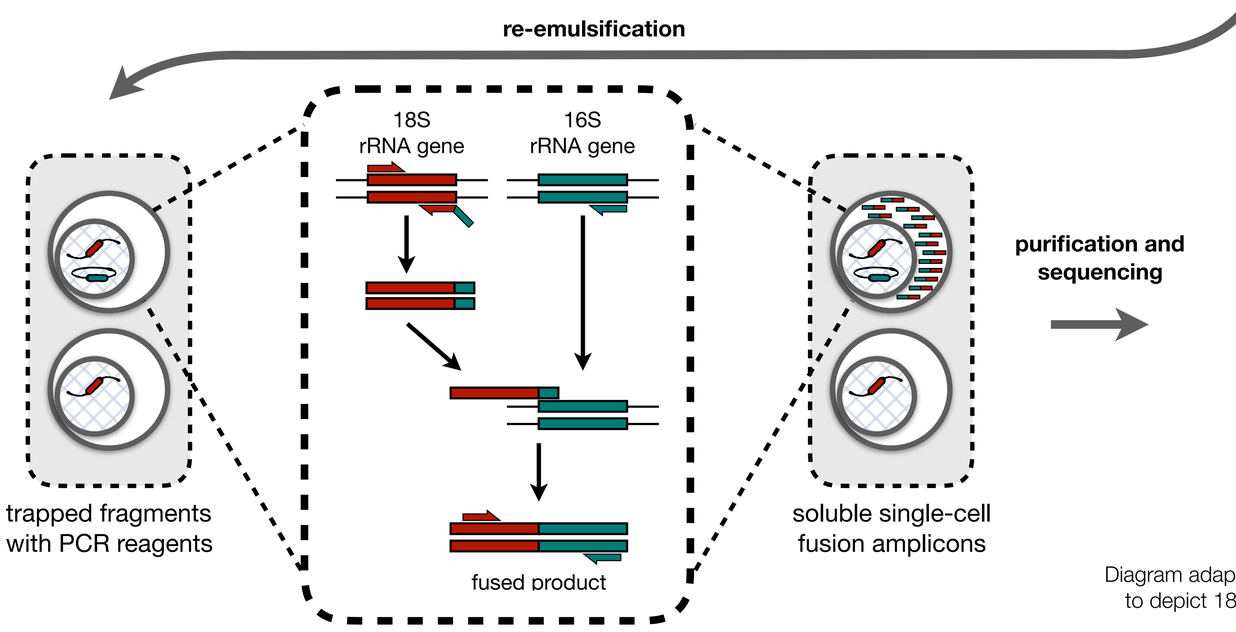


Diagram adapted from ref. 3 to depict 18S-16S linkage

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References

[1]Mitra, A. *et al*. *Defining Planktonic Protist Functional Groups on Mechanisms for Energy and Nutrient Acquisition: Incorporation of Diverse Mixotrophic Strategies*. Protist **167**, 106-120 (2016)
[2]Carvalho, W. F. & Granéli. E. *Acidotropic probes and flow cytometry: a powerful combination for detecting phagotrophy in mixotrophic and heterotrophic protists.* Aquat Microb Ecol **44**, 85-96 (2006).
[3]Spencer, S. J., Tamminen, M. V. et al. *Massively parallel sequencing of single cells by epicPCR links functional genes with phylogenetic markers.* ISME J **10**, 427-436 (2016)

The drawing of the flagellate with mastigonemes has been adapted from an original by Dennis Barthel (CC BY-SA 3.0).