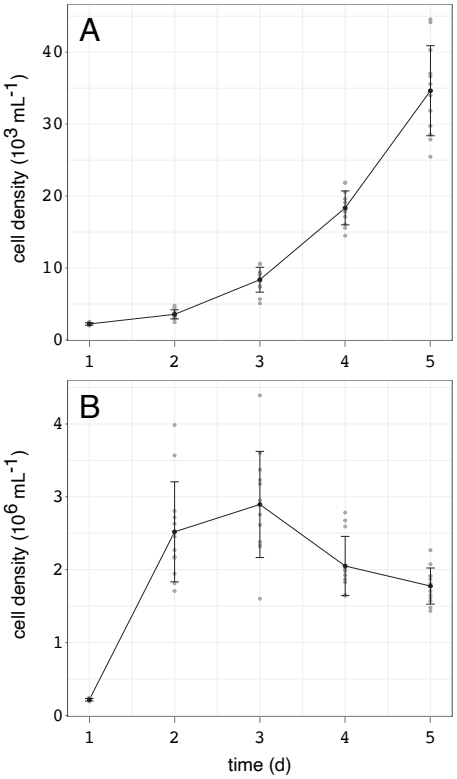

Figure S1. Growth curves of (A) *Ochromonas triangulata*, and (B) the bacterial population present in their culture during the 5 days prior to the experiment. Growth of *O. triangulata* corresponds approximantely to 1 doubling per day.

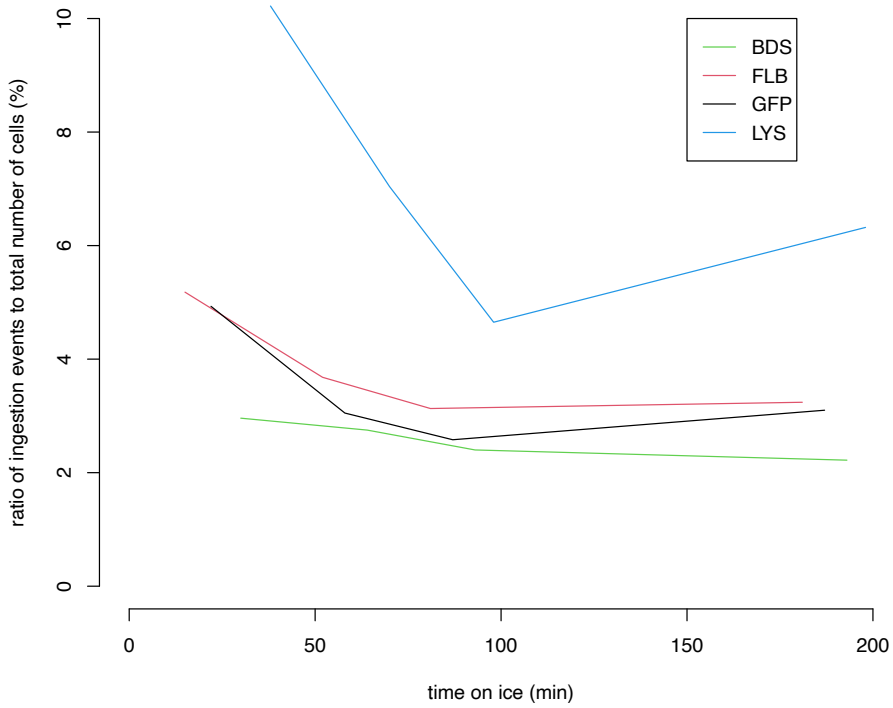

Figure S2. Plots showing the ratios between cells displaying ingestion behavior and the total number of cells of the gated population, as measured using a CytoFLEX flow cytometer. Fluorescently labelled tracers (FLT) used as prey surrogates were (BDS) 1 µm polystyrene beads, (FLB) FLB from *E. coli* cells and (GFP) GFP-expressing *E. coli*, whereas (LYS) refers to food vacuole staining using LysoSensor DND-167. To build this plot, enrichment cultures from natural origin (a lake water sample collected at 2 m depth from oligotrophic lake Långsjön in Björklinge, Sweden, in Oct 2020) were incubated with either three different FLT during 40 min in a way analogous to the one described in the Material and Methods section, or with LysoSensor DND-167 for 10 min after 30 min in darkness at the same temperature. After incubation time, aliquots were collected and placed on ice, and were subsequently examined in a CytoFLEX flow cytometer (see Material and Methods) at increasing timepoints to evaluate cell ingestion behaviour over time while samples remained cooled. As seen in the figure above, no further ingestion was observed after exposure to temperatures close to 0°C.

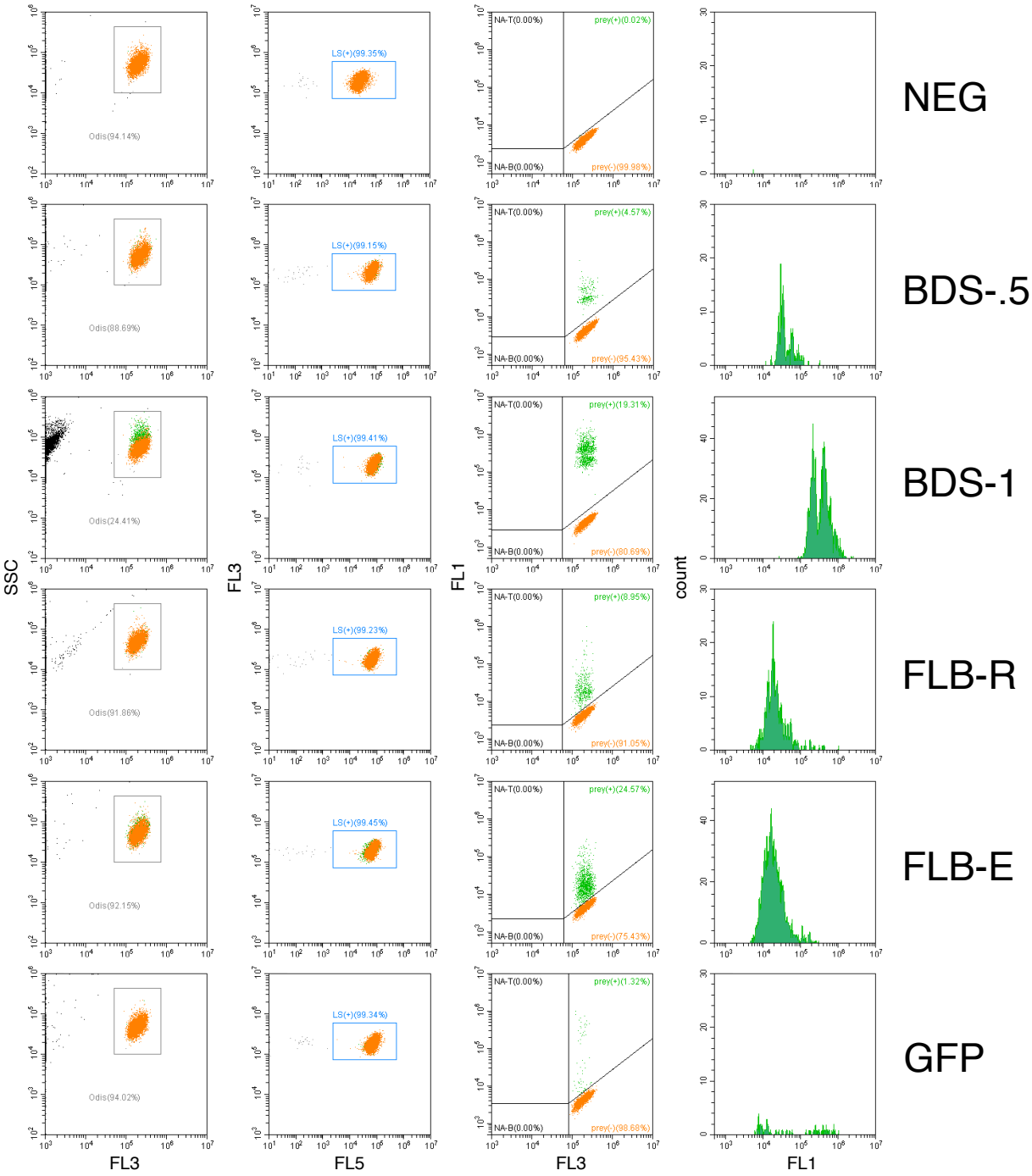

Figure S3. Gating strategy employed to discriminate between actively feeding *Ochromonas triangulata* cells that ingested a fluorescently labelled tracer (FLT-positive) and actively feeding cells that didn't ingest any FLT (FLT-negative). The *O. triangulata* population was identified based on side scatter signal (SSC) and chlorophyll autofluorescence (FL3), and the actively feeding cells subpopulation was determined based on food vacuole fluorescence (FL5 channel) from LysoSensor Blue DND-167. Discrimination between FLT-positive and FLT-negative cells was carried out based on FLT signal in the FL1 channel. The last panel on each row depicts the frequency distributions of all the FLT-positive events along the FL1 channel. Each row corresponds to: (NEG) subsample without the addition of any FLT, (BDS-.5) 0.5 µm polystyrene beads, (BDS-1) 1 µm polystyrene beads, (FLB-R) FLB from Limnohabitans sp. cells, (FLB-E) FLB from E. coli cells, and (GFP): GFP-expressing E. coli. All diagrams in each row correspond to the same replicate, and all replicates displayed here were sampled at 20 min incubation time.

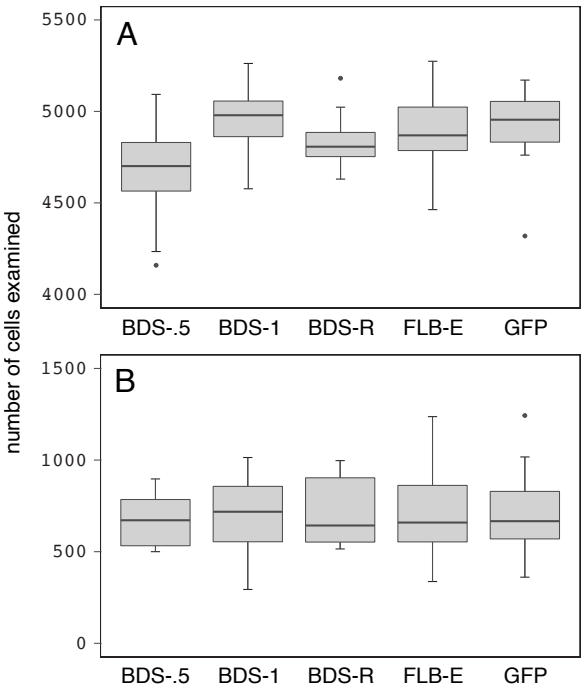

Figure S4. Boxplots representing the distributions of sample sizes per replicate, separated according to prey type, for (A) the number of observations collected via flow cytometry, and (B) the number of observations collected via epifluorescence microscopy. (BDS-.5) 0.5 µm polystyrene beads, (BDS-1) 1 µm polystyrene beads, (FLB-R) FLB from *Limnohabitans* sp. cells, (FLB-E) FLB from *E. coli* cells, and (GFP): GFP-expressing *E. coli*.

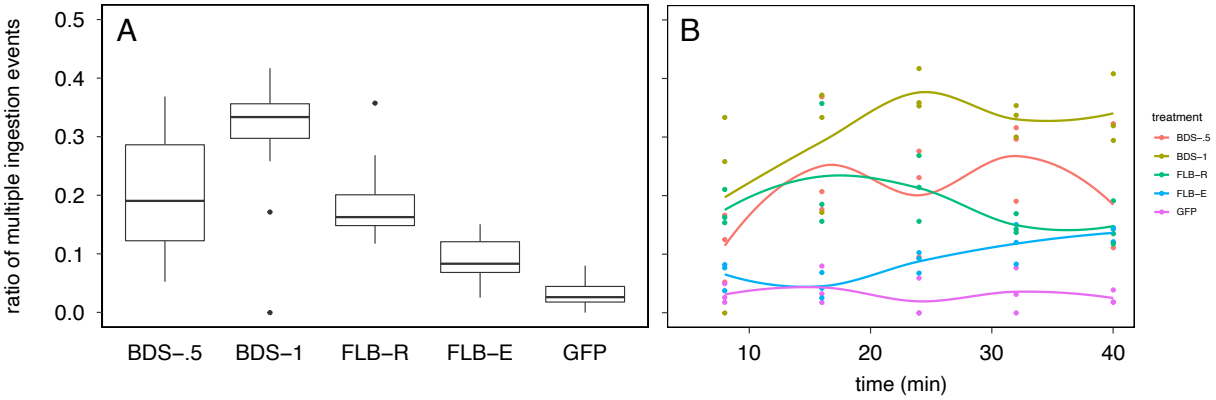

Figure S5. Ratios of multiple ingestion events per cell over the total number of ingestions per replicate. (A) Represented as distributions across fluorescently-labelled tracer (FLT) types. (B) Represented as scatter diagrams against time, showing no apparent relationship between variables. FLT types: (BDS-.5) 0.5 µm polystyrene beads, (BDS-1) 1 µm polystyrene beads, (FLB-R) FLB from Limnohabitans sp. cells, (FLB-E) FLB from E. coli cells, and (GFP): GFP-expressing E. coli.